Addis Ababa Science and Technology University

College of Biological and Chemical Engineering
Department of Environmental Engineering

Optimization of Anaerobic Digestion of Vinasse for the Production of Biogas

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This is to certify that the thesis prepared by Assefa Ayalew, entitled: *Optimization of Anaerobic Digestion of Vinasse for the Production of Biogas* submitted in partial fulfillment of the requirements for the degree of Degree of Masters of Science in Environmental Engineering complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Table of Contents Page

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II. List of Tables Page

III. List of Figures Page

IV. List of Appendices Page

V. Abstract

The increase in energy price and environmental concerns focused attention on the need for industrial process improvement and development of alternative energy sources such as ethanol fuel. Ethiopia, in its GTP 2, has planned to produce 320,268 m^3 of ethanol leaving around 3.8 million m^3 of vinasse as a by-product.,having very high biological oxygen demand (BOD), chemical oxygen demand (COD), and other pollutant properties which necessitate distillery plants to deal with its treatment and reuse. The purpose of this thesis work was to optimize the anaerobic treatment vinasse for substantial decrease of its BOD and COD while producing methane gas and to evaluate potential of vinasse as an alternative source of energy. Vinasse sample was taken from Metehara sugar factory distillery plant. The combined effect of temperature and pH on biogas production and reduction of organic load such as COD, BOD and VS was studied in this work. Accordingly, vinasse samples that were treated at temperature of 35 ^oC and initial pH of 7.25 for hydraulic retention time of 20 days on anaerobic digestion system has produced 34.68 ml of CH4/g COD while COD, BOD and VS concentration were reduced by 64, 76 and 52.77 % respectively. The Composition of biogas was 81 % CH_4, 14 % CO_2, 2 % O_2, 3 % other and 1 PPM H_2S. The minimum amount of biogas production (21.05 ml of CH4/g COD) and low removal efficiency of COD, BOD and VS (30.8, 45, and 25.4 % respectively) were observed at samples treated with temperature of 30 ^oC and pH of 6.5. From the design of UASB reactor, the expected amount of energy generated from anaerobic digestion of vinasse of Metehara sugar factory distillery plant is 1.99 x 10^8 KJ/day, which is equivalent to 10850 KWH.. It was noted that vinasse is a suitable feedstock for anaerobic digestion and can be a good source of alternative green energy and protect the environment from pollution.

Key words: Vinasse, Anaerobic digestion, COD, BOD, Biogas, Temperature, pH

Chapter 1: Introduction

1.1. Background

The Ethiopian government has implemented different programs and plans for the improvement of the well-being of the citizens and the growth of the economy. Some of these includes - Agriculture -Led -Industrialization which is devised towards the renovation or shift from agricultural led to industrialization and the Growth and Transformation Plan (GTP I and II). In line with the GTPs, sugar industries development has been prioritized and is under implementation. As a result, the Federal Democratic Republic of Ethiopia (FDRE) has launched sugar development program to undertake projects such as about 10 new and expansion works on the existing sugar industries across the country with a clear objective of boosting sugar production to satisfy the domestic sugar demand as well as for any possible export in the coming five years. Moreover, sugar production from sugarcane is known for its byproducts that can serve for the proliferation of energy sources and a multitude of industries. However, the country could not harness this potential due to lack of technologies that can be implemented simply with low cost and skilled human resources. Currently, the FDRE has noticed the importance of harnessing this potential that has been used very little so far and therefore has requested several higher education and research institutes in the country to undertake a study on sugar industry by-product utilization for energy and development of bio industries as well as other derivatives.

Ethiopia has been allocating large amount of foreign currency for importing fossil fuel although it has potential for bio-energy resources development such as vinasse, non crop oil plants, organic wastes which can be used for biogas production. In future prediction, however, developing countries will have faced energy supply crisis due to increased oil price and causing global warming, so that attention should be given on the need for development of alternative energy sources such as biogas, bioethanol fuel, etc and improve industrial process [1, 72]. In addition, pressing economic constraints and environmental regulations have placed a demand for increased productivity and diversification of the industrial plant byproducts portfolio. Segregating the organic wastes from sugarcane industries such vinasse and other less valuable fractions for use as energy like biogas and biofuel, thus creating value-added products, appeals to present productivity demands, as well as create comfortable working environment for the sugar industries employee.

Sugar cane distillery waste disposal improvements are strongly needed, as evidenced by vinasse, or spent wash, which is the liquid residue left after distillation of alcohol having high organic load and strong acid [72]. In addition, recent breakthroughs research findings in enzyme technology and processing were radically changing the viability of ethanol and other by-products as a transportation fuel from different biomasses including organic wastes [14].

Sugar Corporation is working vigorously to raise the nation's current sugar production capacity remarkably so that the nation will greatly benefit from the sector. According to the survey conducted at a national level on the water resource and canal development opportunities, it is proved that the country has a potential of more than 500,000 hectares of land suitable for sugarcane plantation. The upper and lower areas of Beles River, areas of South-West of Lake Tana called Upper Dinder, areas along Tekezzie River and its tributaries around Welkayit and Humerra, valleys of Anger River - Negiesso, central Genallie River and Barro-Gillo rivers of Gambella are among some of the areas suitable for sugar cane plantation. Based on the above survey the Corporation is currently building ten new sugar factories, among these Tendaho Sugar Factory, Kessem and Arjo-Didessa sugar factories have entered in to regular production in different months of 2015. The Corporation has undertaken Arjo Didessa Sugar Factory which had been under the ownership of a Pakistan company called Al-Habesha P.L.C since mid of 2012[11]. It is well known that industrial processing of sugarcane results in the generation of large amounts of wastes such as bagasse, ashes, vinasse, and other liquid and gaseous residues. The retrieval of energy and the production of diverse products, including the reuse of wastes, would be an application of the currently important concepts of bio refinery and sustainability for the Ethiopian bio-industry. Thus, it is timely to undertake researches on the development of alternative technologies such as biogas technologies using vinasse feedstock.

Australia, Brazil and other countries have been applying untreated vinasse to fertilize sugarcane fields for many years [41]. According to Turner et al [42], the irrigation of sugarcane fields with vinasse started in the 1920s. However, direct using of vinasse for fertilizer can generate problems on water quality and soil microbes, due to its high COD, low pH, and high concentrations of various constituents. In Ethiopian context, however, the trend on vinasse handling indicated that with the exception to utilize a little for bio-compost mixed with filter cake, most vinasse were

disposed into the nearby water bodies. Therefore, it is quite important to develop well optimized biogas technology for a better way of treating and disposing vinasse.

Ethiopian Sugar Corporation has planned to integrate ethanol production unit along the factories planned to be built. The amount of ethanol to be produced from current operation and future projects are approximately 320,268m^3 per year [11]. These distillery plants are expected to generate around 3.8 million m^3 of vinasse. Currently, Metehara and Fincha sugar factories have ethanol plant in operation, while there is also a plan to establish additional ethanol manufacturing plants from the main by-product called molasses.

Metehara Sugar factory is currently producing 350, 000 liters of vinasse to produce 50000 liters of ethanol per day [11, 13]. There is vinasse concentration unit planned to minimize this volume, although the unit was not operational during my visit. From personal observation, vinasse was being used to make bio-compost along with filter cake. In addition, attempt was being done to mix some amount of vinasse (5 %) with water for fertilizing sugar cane irrigation. There is still surplus vinasse to be dumped to water bodies especially when bio-composting plant has got technical problem.

Vinasse is a dark brown wastewater produced in large amounts in ethanol production from sugar cane processed. The fermentation of sugar cane and the subsequent distillation of ethanol generate between 10 and 15 liters of vinasse per liter of ethanol produced [1, 2, 5]. Vinasse contains mainly water, organic minerals, suspended solids and other pollutants and high acid. Apart from high organic content rates, vinasse also contains nutrients, such as nitrogen (1,660-4,200 mg/l), phosphorus (225-3,038 mg/ L) and potassium (9,600-17,474 mg /L).[3] It is characterized by very high chemical oxygen demand (COD) (60000-200000 mg/l) and biochemical oxygen demand (BOD) (25000-75000mg/l), and pH of 3.7 – 5. [3]. Therefore, disposing such organic load of vinasse to the water bodies might causes the proliferation of microorganisms that deplete the oxygen dissolved in the water, kill aquatic animals and plants, and make contaminated water bodies more difficult to be used as sources of potable water. In addition, the discharge of vinasse in water bodies releases an unpleasant odor due to oxidation and contributes to serve as pest breeding site that can disseminate diseases such as malaria, amebiasis, and schistosomiasis [3] by absence of natural predators and/or vectors. It is not

necessarily the volume of vinasse, but restrictions for effluent's physical and chemical composition such as biochemical oxygen demand (BOD) discharge by current environmental laws and regulations that seems to present the biggest challenge to the profitable use and disposal of vinasse. Therefore, before damping to the nearby water body, treatment of vinasse is very essential and of great importance to the community and environment at all. Of the various treatment methods for distillery wastewater, anaerobic digestion has gained wide acceptability due to recovery of gas of high calorific value (methane) in the anaerobic step of the treatment as well as relatively non offensive sludge suitable for use as a bio fertilizer. It is also reported that anaerobic treatment results in substantial reduction of BOD, COD and other pollutants [2].

Furthermore, sugar and ethanol processing are energy intensive and requires steam and electricity. Based on the analysis of the existing situation of the country with international scenario, the sugar industries of Ethiopia to become competitive in the international market, they need to have an effective cogeneration scheme. Mass and energy balance computation showed that old sugar factories with inefficient cogeneration plant crushing cane of about 14 % fiber consume the entire bagasse and in some cases even require additional fuel sources such as furnace fuel [13]. Thus, the present research was initiated to develop well optimized alternative energy technology to utilize vinasse for biogas production and vinasse sludge for composting.

The availability of biogas as alternative energy source is used to avoid the cost that could be incurred by purchasing furnace fuel. Furthermore, it is possible to avail surplus bagasse that can be used as raw material for paper production, other fibrous products or for production of extra electric energy in condensing mode turbines for sale to the grid [13]

Anaerobic digestion using vinasse as substrate for vinasse treatment and biogas production has been performed by UASB successfully in Brazil [69]. Upflow Anaerobic sludge blanket reactor (UASB) has a bottom sludge bed, dense and granular anaerobic biomass. Mixing is provided by the upflow velocity and biogas generation [12]. UASB is designed for higher COD loading (5 – 20 kg/m^3.day) [12]. As vinasse is known for its high organic load UASB is preferred reactor for its anaerobic treatment. Over 500 UASB units have been built in the world for treating high BOD industrial waste waters such as, waste waters from distilleries, Diaries, pulp mills, pharmaceutical units, starch maize units, textile units and tanneries [70].

Therefore, the purpose of the present study was to optimize anaerobic treatment of vinasse for substantial methane production while decrease BOD, COD and neutralize the acid and to evaluate potential of vinasse as an alternative source of energy through the burning of biogas produced by anaerobic digestion process in Metehara Sugar factory.

1.2. Statement of the Problem

Molasses based distilleries are among the industries having high polluting effect to the environment due to large organic load associated with their discharges. Currently, most potable alcohol distilleries and ethanol plants in Ethiopia are discharging their effluents to adjacent rivers without proper treatment. As consequence, they are facing strong complaints from the local communities and environmental protection bodies.

One of the biggest problems in Metehara sugar factory's Ethanol plant is the disposal of acidic and concentrated organic effluent (360 m^3/day) generated from distillation section. Although an attempt is being done to use vinasse as fertilizer by mixing with water, this experience is questioned due to its negative impact on soil fertility [42]. Since vinasse increases the temperature of the receiving water body and reduces dissolved oxygen, its direct discharge in rivers and lakes causes serious aquatic ecosystem pollution problems [5]. Vinasse has high concentration of P and N nutrients that can cause eutrophication in water bodies. Vinasse's acidity also makes possible the dissolution of metals in the water, while its dark brown color was blocked sunlight penetration so that hinders photosynthesis of riverbed plants and is therefore harmful to aquatic life. If vinasse is disposed to water bodies, not cooled before, temperature of water bodies can increase, so that it can disturb the aquatic organisms' activity. [52]

Disposal of vinasse directly to the soil has led to soil salinity and sodicity due to the presence of soluble salt in vinasse, consequently, soil structure become poor and decreased fertility [5, 43, 72]. In addition, vinasse was considered highly toxic to animals and plants which has a great deal of negative impact to the downstream of Afar and Kereyu communities where their day to day lives are associated with Awash River. In Ethiopia, although the impact of disposed vinasse on the environment was not well studied, presence of phenolic compounds in vinasse interfere the degradation process of vinasse. Oxidation bacteria cannot degrade the phenolic compound, so if vinasse is disposed in the environment, it will be difficult to be degraded [51]. Instead, sugarcane vinasse may be contribute significantly for greenhouse gas (GHG) emission to the atmosphere

that might be resulted from aerobic and anaerobic decomposition of the organic matter in vinasse that occurs during transportation, temporary storage or even after application to soil. Recent study has showed that the application of vinasse to sugarcane fields in Brazil resulted in significant increases in the emissions of GHG, especially N_2O [4]. Metehara Sugar Factory was applied mixed filtercake-vinasse compost to sugarcane plantation and the nutrient content of the compost, rate of application, and its effects on sugar yield and soil properties were studied[53]. However, utilization of vinasse for biogas production and the slurry of vinasse biogas for fertilizer and compared the nutrient content with filter cake vinasse compost were not well known.

The other problem associated with the distillery plant in Metehara is supply of electricity for the operation of the plant. Due to lack of electricity generated in steam power turbines, the ethanol plant is getting its electric power need (600 KWH) from national grid. The power interruption of the national grid may cause industrial process which can cause production loss. Thus recovered methane gas from anaerobic treatment of vinasse can substitute the power demand and interruption. The generated methane can also be used as direct fuel for steam generation in boilers along with bagasse, which in turn saves bagasse consumption. The slurry produced from the vinasse biogas digester can also be used for bio-fertilizer so that reduce fertilizer expenses.

1.3. Hypotheses /Questions

- What are the characteristics of the disposed vinasse from Metehara Sugar factory? Does it meet environmental standards of Ethiopia?
- What are the optimum temperature, pH and concentration of vinasse for high production of methane while reducing carbon dioxide?
- What is the efficiency of anaerobic digestion process on the quality and quantity of biogas produced and on the reduction of environmental burdens of vinasse?
- Can the recovered biogas characteristics be used as alternative fuel source for the distillery?

1.4. Objectives

1.4.1. General Objective

The general objective of this research was anaerobic treatment of vinasse by optimization of mesophilic temperature and pH for biogas production while decreasing organic pollutants and evaluation of vinasse potential as an alternative source of renewable energy

1.4.2. Specific Objectives

The specific objectives of the research topic were:

- Characterization of the vinasse generated from the distillery of Metehara Sugar factory
- Identification of optimum operating conditions (temperature, pH) for maximum production of biogas.
- Evaluation of the biogas generated from vinasse anaerobic digestion as an alternative source of energy.
- Evaluation of the efficiency of anaerobic digestion sludge treatment process by comparing with Ethiopian standards.
- Design of Up flow Anaerobic Sludge Blanket (UASB) reactor for vinasse treatment

1.5. Significance of the Study

The study will try to answer the problems associated with the optimization of anaerobic digestion by identifying the optimum parameters such as temperature, pH and feedstock concentration. The result of the present thesis will be beneficial to Metehara Sugar Factory in particular and Ethiopian sugar corporation in general by reducing the environmental constraints of its distillery waste while producing high amount of biogas for their energy demand and producing biofertilizer from the slurry of the biogas digester if implemented, and can be good demonstrating site for the community. Specifically the result will help in determining operating parameters of anaerobic digestion related to distillery wastes so that improve the efficiency of the biogas plant through increasing the production of methane gas while reducing carbon dioxide. In addition, the result of the present study will serve as a baseline data for further studies on anaerobic digestion process of distillery wastes and for policy makers. Furthermore, alcohol producing companies can use the result of this thesis to develop their own anaerobic digestion plant to reduce the environmental burdens of their waste while producing biogas for their energy demand and create huge improvement in the public relation image of their organization.

1.6. Scope of Work

The scope of this thesis work was optimization of pH (6.5 – 8) and Mesophilic temperature (28 – 42 ^{o}C for vinasse anaerobic digestion using design expert response surface methodology central composite design. Effect of indicated pH and temperature on biogas & methane yield and reduction of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Volatile Solids (VS) was studied.

Chapter 2: Literature Review

2.1. Over view of Anaerobic Digestion

Anaerobic treatment can be defined biochemically as the conversion of organic compounds into Carbon dioxide, methane and microbial cells (sludge), in the absence of free or molecular oxygen [40].

The various chemical reactions brought about by bacteria are due to the activity of enzymes or "ferments" elaborated by the bacterial cells. Test of different bacteria indicate that they are about 80% water and 20% dry material, of which 90% is organic and 10% inorganic. An approximate formula for the organic fraction is $C_5H_7O_2N$ [1].

To continue to reproduce and function properly, an organism must have (1) a source of energy, (2) carbon for the synthesis of new cellular material, and (3) inorganic elements (nutrients) such as nitrogen, phosphorus, sulfur, potassium, calcium and magnesium. Organic nutrients (growth factors) may also be required for cell synthesis. Two of the most common sources of cell carbon for microorganisms are organic matter and carbon dioxide. The energy needed for cell synthesis may be supplied by light or by a chemical oxidation reaction [1].

Required organic nutrients, known as "growth factors," are compound needed by an organism as precursors or constituents of organic cell material that cannot by synthesized from other carbon sources. Among the major growth factors are amino acids, purines and pyrimidines, and vitamins

Process Steps of Anaerobic Digestion

The process of biogas formation is a result of linked process steps, in which the initial material is continuously broken down into smaller units. Specific groups of micro-organisms are involved in each individual step. These organisms successively decompose the products of the previous steps. The simplified diagram of the AD process, shown in Figure 2.1, highlights the four main process steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [40].

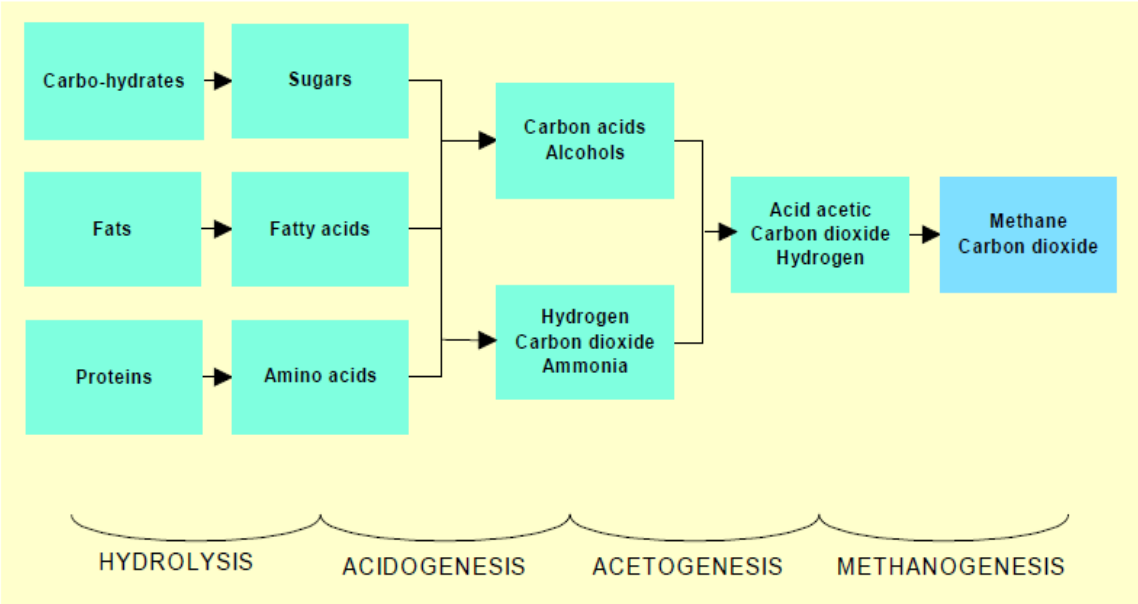

Figure 2.1: Stages of anaerobic digestion

Hydrolysis

Hydrolysis is theoretically the first step of AD, during which the complex organic matter (polymers) is decomposed into smaller units (mono- and oligomers). During hydrolysis, polymers like carbohydrates, lipids, nucleic acids and proteins are converted into glucose, glycerol, purines and pyridines. Hydrolytic microorganisms excrete hydrolytic enzymes, converting biopolymers into simpler and soluble compounds as it is shown below:

$$\text{Lipids} \xrightarrow{\text{lipase}} \text{fatty acids, glycerol}$$

$$\text{Polysaccharide} \xrightarrow{\text{cellulase, cellobiase, xylanase, amylase}} \text{monosaccharide}$$

$$\text{Proteins} \xrightarrow{\text{protease}} \text{amino acids}$$

A variety of microorganisms is involved in hydrolysis, which is carried out by exoenzymes, produced by those microorganisms which decompose the undissolved particulate material. The products resulted from hydrolysis are further decomposed by the microorganisms involved and used for their own metabolic processes.

Acidogenesis

During acidogenesis, the products of hydrolysis are converted by acidogenic (fermentative) bacteria into methanogenic substrates. Simple sugars, amino acids and fatty acids are degraded into acetate, carbon dioxide and hydrogen (70%) as well as into volatile fatty acids (VFA) and alcohols (30%).

Acetogenesis

Products from acidogenesis, which cannot be directly converted to methane by methanogenic bacteria, are converted into methanogenic substrates during acetogenesis. VFA and alcohols are oxidised into methanogenic substrates like acetate, hydrogen and carbon dioxide. VFA, with carbon chains longer than two units and alcohols, with carbon chains longer than one unit, are oxidized into acetate and hydrogen. The production of hydrogen increases the hydrogen partial pressure. This can be regarded as a waste product of acetogenesis and inhibits the metabolism of the acetogenic bacteria. During methanogenesis, hydrogen is converted into methane. Acetogenesis and methanogenesis usually run parallel, as symbiosis of two groups of organisms.

Methanogenesis

The production of methane and carbon dioxide from intermediate products is carried out by methanogenic bacteria. 70% of the formed methane originates from acetate, while the remaining 30% is produced from conversion of hydrogen (H) and carbon dioxide (CO_2), according to the following equations:

$$\textit{Acetic acid} \xrightarrow{\text{methanogenic bacteria}} \textit{methane} + \textit{carbon dioxide}$$

$$\textit{Hydrogen} + \textit{carbon dioxide} \xrightarrow{\text{methanogenic bacteria}} \textit{methane} + \textit{water}$$

Methanogenesis is a critical step in the entire anaerobic digestion process, as it is the slowest biochemical reaction of the process. Methanogenesis is severely influenced by operation conditions. Composition of feedstock, feeding rate, temperature, and pH are examples of factors influencing the methanogenesis process. Digester overloading, temperature changes or large entry of oxygen can result in termination of methane production.

The anaerobic decomposition of organic matter is a three-stage reaction: (1) hydrolysis of the organic material into soluble organic compounds, (2) acetogenesis, or conversion of soluble organics to volatile fatty acids (mostly acetic acid); and (3) methanogenesis, or conversion of the volatile fatty acids into methane [6].

In the digester the bacterial culture carries out the conversion in accordance with the following Stoichiometric equation [6]

$$C_nH_aO_b + \left(n - \frac{a}{b} - \frac{b}{2}\right) H_2O \longrightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{8}\right) CO_2$$

It is important to note that methane bacteria can only use a limited number of substrates for the formation of methane. Currently, it is known that methanogens use the following substrates: CO2 + H2, formate, acetate, methanol, methylamines, and carbon dioxide.

2.2. Main Factors Affecting Anaerobic digestion

The environmental parameters controlling anaerobic digestion are temperature, pH, buffering capacity and volatile fatty acid concentration.

2.2.1. Temperature

The three temperature ranges under which anaerobic digestion can occur are psychrophilic, Mesophilic and Thermophilic [15].

Table 2.1: Possible anaerobic digestion temperatures

Temperature	Range
Psychrophilic	< 25^{o}C
Mesophilic	25^{o}C – 45^{o}C
Thermophilic	45^{o}C – 70^{o}C

Each temperature range supports a specific type of methanogenic bacteria that are sensitive to temperature fluctuations, as shown in Table 2.1. Temperature is one of the most critical operation parameters during anaerobic digestion. While digesters of all configurations have been run successfully under psychrophilic (<25°C), mesophilic (25-55°C) and thermophilic (>55°C) conditions, the operational considerations with regard to maintaining microbial community stability for each situation are different. Psychrophilic digestion has been possible when processing manure and while it requires almost no energy input, the amount of biogas produced trails mesophilic and thermophilic operations. The growth of methanogenic bacteria is slower at temperatures cooler than 25°C, and significant acclimatization of the seed inoculum has been required to prevent a long lag phase in methane production during reactor start-up [16, 18]. Mesophilic digestion is the most common configuration, and has a reputation as being the most stable in term of consistent biogas production. Thermophilic digestion has the most potential to

maximize biogas production; however it has been shown that maintaining an equilibrium between acidogenic and methanogenic activity is easier at mesophilic compared to thermophilic temperatures [17]. Additionally, maintaining the reactor at thermophilic temperatures requires significantly more energy input into the system, decreasing the new energy gain of the reactor.

Figure 2.2, shows how similar levels of biogas or methane can be produced in shorter periods of time under thermophilic conditions (15 – 20 days) compared to mesophilic conditions (30 – 40 days) and psychrophilic conditions (70 – 80 days) [15].

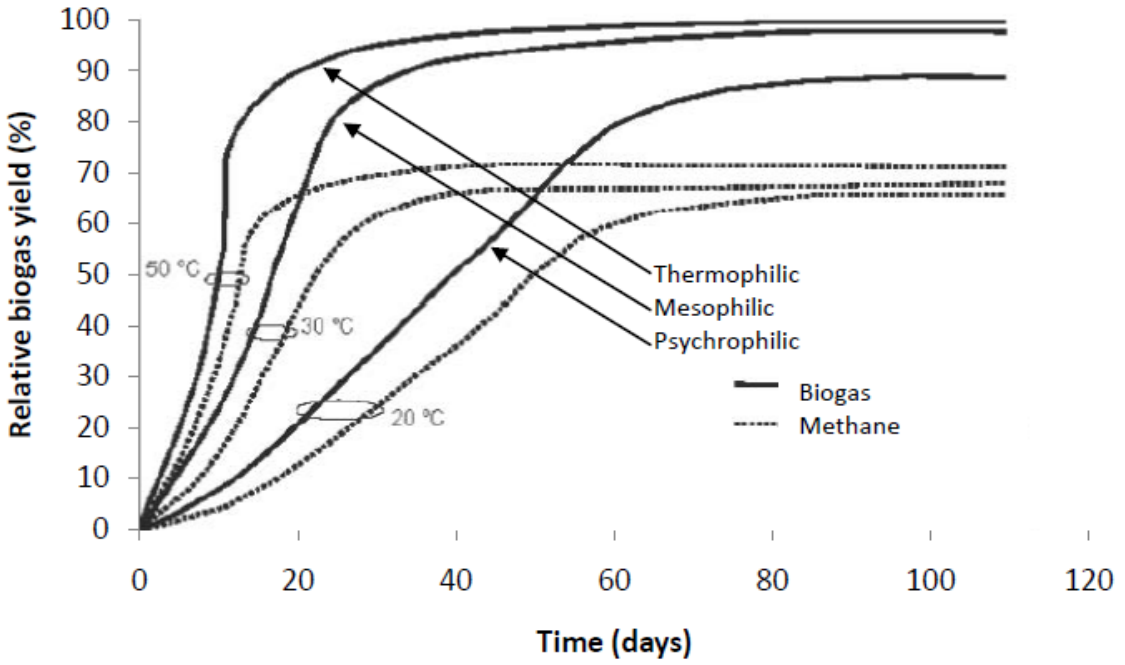

Figure 2.2: Temperature effect on biogas yield (Hecht M. 2009)

Thermophilic organisms have the fastest growth rate which allows engineers to design smaller systems with shorter hydraulic retention times, but the solubility of some gases (NH3, H2, CH4, H2S and VFA) also increases with temperature and can have a negative impact on the system if the gas has an inhibitory effect [15] Thermophilic conditions have shown to improve digestibility and substrate utilization, but the microbes are more sensitive to temperature fluctuations [15]

In additional to the flow of input material, level of mixing, and inoculation protocol, temperature is one of the most critical operation parameters during anaerobic digestion. While digesters of all configurations have been run successfully under psychrophilic (<25°C), mesophilic (25-55°C)

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2.2.2. PH

The pH requirements that are optimal for the growth of microorganisms vary for different groups of microorganisms. Low pH (5 to 6.5) is generally optimum for the growth of fermentative bacteria, which are responsible for enzymatic hydrolysis of polymers to monomers and subsequent conversion to acids. Neutral pH is optimum for the growth of methanogens. Methanogens are known to be more sensitive to pH changes than the fermentative bacteria. pH is also an important parameter since the toxicity of intermediates such as ammonia and SCFA (short chain fatty acids) is a function of the pH of the system. In general, a pH range between 6.8 and 8 is suggested to be an optimum condition for operating biogas plants. pH values between 6.7 and 7.4 are known to optimize methane formation, whereas disruptions in digester performance have been experienced when the range drops below 6 [18, 20]

2.2.3. Buffering Capacity

The buffer capacity of the system that is expressed in terms of alkalinity is an important parameter that provides resistance to significant and rapid changes in pH. A kinetic uncoupling of acid producer and consumer is usually associated with an accumulation of SCFA and thus, pH decreases in less buffered system [19]. Since pH reduction is associated with process imbalance, alkalinity or pH is used as a tool for monitoring process imbalance. However, in highly buffered

systems, pH changes can be small, even when the process is extremely stressed, suggesting pH is less important to indicate process imbalance in this condition [19]. This shows that the use of pH as a tool for monitoring process depends on the specific reactor system and operating condition.

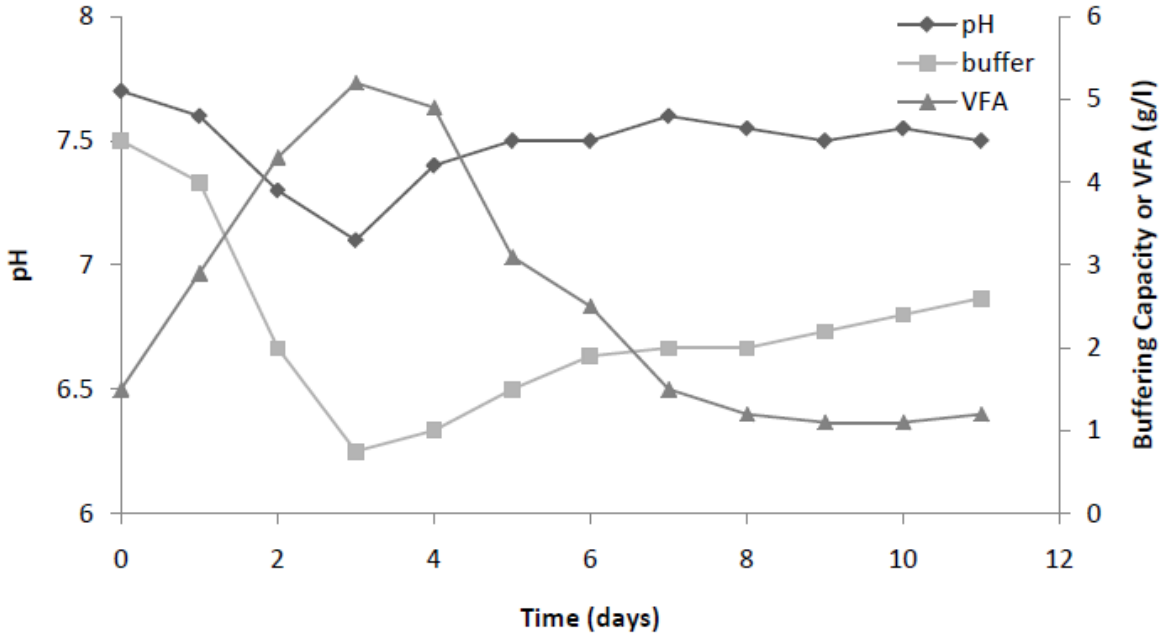


Figure 2.3: Relationship between pH, Buffering capacity and VFAs (Hecht M. 2009)

2.2.4. Volatile Fatty Acids

Figure 2.3, also shows the relationship between volatile fatty acids (VFAs), pH and buffering capacity. VFAs are the intermediate products of acidogenesis and will accumulate if the symbiotic relationship between acetogenic and methanogenic bacteria is sacrificed. The accumulation of VFAs causes a subsequent drop in pH which in turn creates a toxic environment for methanogens (pH<6) [19, 21]. The VFA accumulation shown above corresponds with a drop in pH and the consumption of buffering capacity to correct both VFA and pH levels during digester operation.

Monitoring fluctuations of the VFA levels in a specific digester is the most telling sign of process instability, whereas comparing VFAs between digesters provides little information due to variations in input material and microbial response [19, 25]. Some VFA accumulations are less concerning than others. For example, acetate feeds methane production directly, so its

contribution to the VFA profile is less concerning than say propionate or butyrate which require degradation to acetate before they are available to methanogens. Increases in acetate have been shown to increase metabolic activity and methane production, whereas increases in propionate have indicated low metabolic activity and slow process stabilization [22, 25]

2.2.5. Substrate selection

Biogas production depends heavily on the substrates entering anaerobic digestion systems. A substrate's chemical and physical properties affect the ability of microbes to convert it into methane. Figure 2.4, shows the biogas yield potential of various substrates. Substrates with high caloric values and simple nutrient structures have much higher biogas potentials than watery substrates with tightly bound nutrients. In grasses and vegetables, for example, complex carbohydrate structures like cellulose, hemi-cellulose and lignin bind nutrients and thus degrade very slowly or not at all in anaerobic digesters. Refined fats and carbohydrates, on the other hand, exhibit higher biogas potentials because microbes can easily access and degrade the high energy nutrients [23].

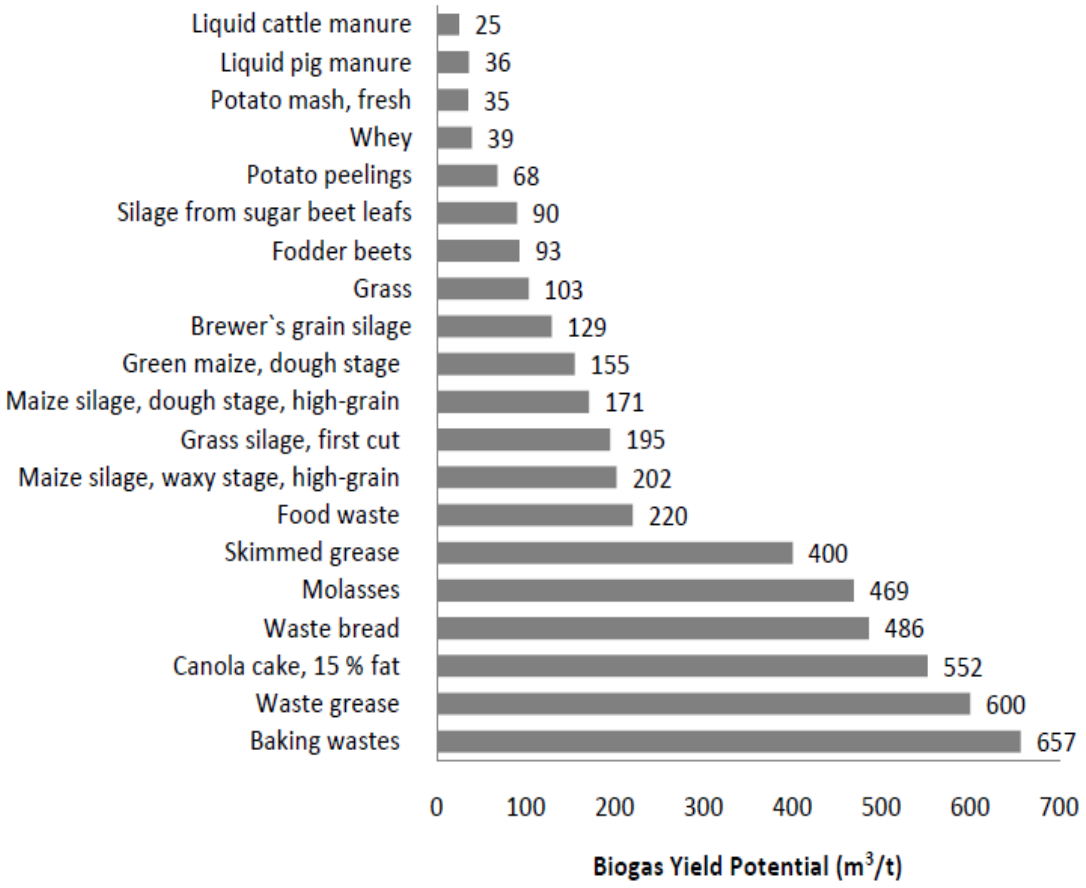


Figure 2.4: Biogas potential of different substrates based on Effenberger, M. (2010)

Every input for anaerobic digestion has its own biodegradation characteristics. Each will generate different volumes of biogas, require different environmental considerations and respond differently to engineering techniques [23, 18]

For the most part, higher concentrations of organic matter in a substrate correspond to increased biogas production. The total and volatile solids content of a substrate are important parameters to be determined before BMP analysis for biogas and methane potential. Total solids content (TS) effects the operation of an anaerobic digestion system. Volatile solids (VS) are the organic fraction of a material that could potentially be converted into biogas. Substrates with high volatile solids to total solids ratio (VS/TS) are expected to produce more biogas per volume of substrate because there is a greater fraction of material available for the microbes to convert into biogas. [23, 18].

2.2.6. Organic loading rate (OLR)

Organic loading rate (OLR) represent the amount of feed added into a digester per unit of time. Depending on substrate, temperature and reactor design, different range of OLR are employed. Typical well-functioning thermophilic digester can be loaded in the range of 4-5 kg VS $m^{-3}d^{-1}$ whereas mesophilic digester has a load of 2-3 kg VS $m^{-3}d^{-1}$[18, 19].

An accidental increase in organic loading is the most common disturbance, which could lead to process instability and process failure in the worst case [19]. Depending on the substrate, parameters such as VFA and hydrogen concentration have been suggested as tools for monitoring process imbalance so that corrective action can be employed before the process collapses. Hydrogen and SCFA have been suggested as a good parameter for a digester treating carbohydrate rich substrate. Hydrogen closely follows SCFA accumulation in this digester [19]. For a digester treating sewage sludge and rape seed oil, the concentration ratio of volatile fatty acids to calcium acted as an early warning indicator [24]

2.2.7. Hydraulic retention time (HRT)

The hydraulic retention time (HRT) is a term commonly used to represent the statistically average residence time of the soluble substrate in the digester. The HRT, which depends on the characteristics of feedstock, reactor design temperature of the digester and environmental conditions, should be long enough to allow metabolism by organisms for the degradation of organic material to biogas. For slowly degradable substrates, the HRT is normally longer to allow the solubilization of the organic material efficiently and in this case, hydrolysis is considered as a rate limiting step. Continuous stirred tank reactor (CSTR) is operated at longer HRT (10-60 days). [18].

It is defined as the active digester volume, VR, divided by the volume of substrate, Vsubstrate, fed per unit time, t.

$$HRT = \frac{VR}{Vsubstrate/t} \qquad \text{..... 1.1}$$

The volume of the digester should allow input materials to be converted into biogas before exiting the system. The goal is to keep material in the digester as long as it is producing biogas, but to remove the material once microbes have used the majority of nutrients from it. Additionally, the growth rate of methanogens in a digester must remain faster than the removal rate of effluent from the digester in order to avoid wash out situations [26].

Organic loading rate (OLR) and hydraulic retention time (HRT) should be balanced for efficient operation. As shown below, biogas productivity increases as OLR increases up to a critical level. Biogas yield per kg of volatile solids increases as HRT gets longer and volatile solids are used up. The point at which the OLR and biogas productivity is optimized does not correspond to the maximum biogas yield per kilogram of volatile solids, but a continuous system designed at this HRT and ORL takes advantage of the time period where the rate of biogas yield is the greatest.

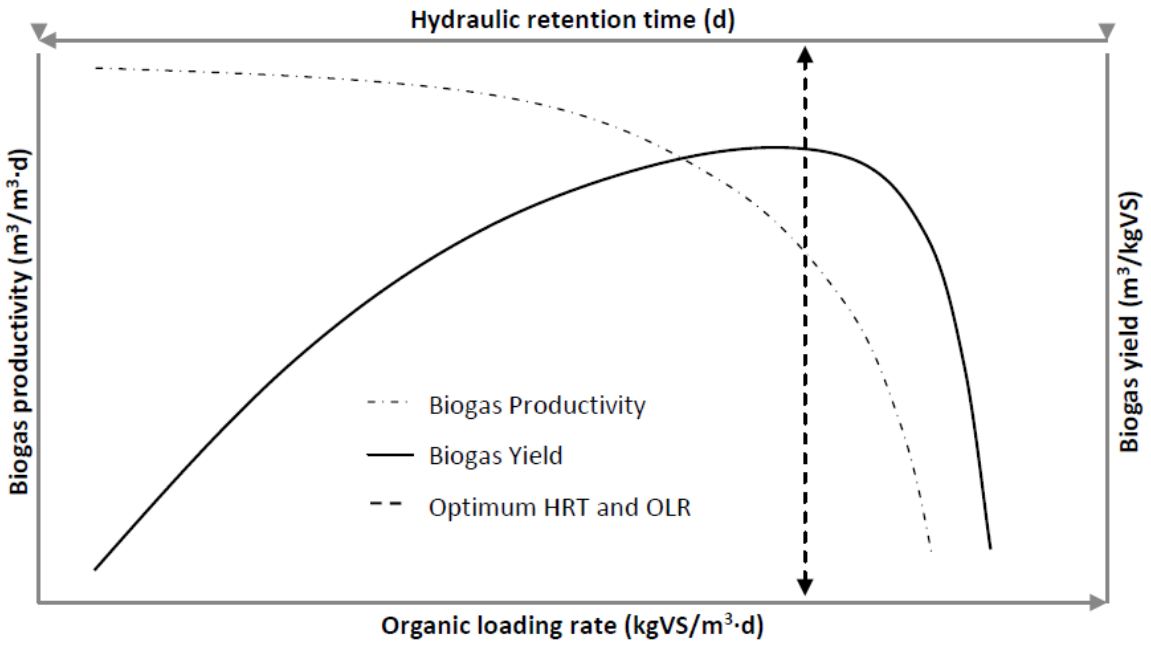


Figure 2.5: Balancing ORT and HRT (Hecht M. 2009)

2.3. Anaerobic digester design and operation

Reactor design and operation are important considerations for maximizing process efficiency. Many different configurations have been used successfully, including incorporating single or multiple reactor cores, closed batch or continuous organic loading, and nutrient or biomass recycling [28]. There are many factors to consider when implementing an anaerobic system for waste processing in order to maximize methane production and the breakdown of organic material while minimizing the retention or processing time required. The earliest designs for anaerobic digesters including the fixed dome, floating cover and balloon type reactors could be adapted for continuous flow or batch operation, required a long hydraulic retention time, and had no active mixing of the material inside the reactor [27]. As the microbial and biochemical dynamics were studied in greater detail, more complicated reactor designs were constructed to maximize the degradation of organic material and methane production.

Batch operation is the most simple reactor configuration as input material is loaded into the reactor to begin the process, the reactor is sealed, and biogas is continually siphoned off as it is produced. In this design, the biochemical steps of methanogenesis occur in sequence, beginning with an outgrowth of hydrolytic bacteria, followed by acidogenic and acetogenic bacteria, and finally methanogenic bacteria. While this is more likely to result in the maximal production of each class of microorganism as they frequently consume their desired substrate until depletion, the retention time is often longer compared to other reactor designs. It is also difficult to accurately model batch digesters and predict their future performance as their inputs and microbial community compositions can undergo significant change between runs [17]. As the start-up phase of the digestion process is often the most time-consuming and least productive part of the process, the fact that this step is repeated every time the reactor is loaded reduces system efficiency. However, the complete change-over in the reactor does allow for more flexibility in the amount and composition of the input being digested, without the concern of disrupting a steady state microbial community.

One of the other more common anaerobic reactor designs is a continuous flow system, with organic material continuously loaded, and biogas and digestate continuously removed. In this reactor design, all steps of the methanogenic pathway are happening concurrently. During the

start-up phase of the system, the flow rate of organic material is gradually increased, allowing for the microbial community to reach a steady state, whereby methane is produced and there is little to no accumulation of volatile fatty acids in the system indicating the activity of the acetogenic community is approximately equal to the metabolic activity of the methanogenic population [17]. Once this steady state is achieved, the organic loading rate is constantly adjusted to maintain equilibrium between these two biochemical processes. Monod-type modeling of microorganism growth rates has been used to quantify and predict microbial community behavior in the reactor, although the requirement to base the model on the microorganism activity that is rate-limiting in the process may lead to misleading results if the community composition and efficiency at different stages undergoes any type of change [17]. While this is a fairly simple reactor system to construct and operate, there are not many ways to easily adjust the system if performance begins to lag. Also, the operating conditions that are conducive to a reactor operating at steady state are likely not the maximal activity of the microorganisms present in the reactor, but a compromise for each so that neither is being completely inhibited.

To increase the stability of the microbial community in continuous-flow reactors, immobilization of the microbes has been examined. Up-flow anaerobic sludge blanket (UASB) reactors select for consortia that are naturally biofilm-forming, which develop into large sludge granules and form a suspended layer in the reactor. The use of synthetic materials as microbial supports have also been tested and found to reduce microbial washout [29]. While these techniques can help retain microorganisms that may otherwise be lost during high flow-rate operation, the diversity of these digester communities is lower, and may be less able to respond to variations in input material, temperature or pH [30].

More recently, the need to try and optimize the different biochemical steps in the methanogenesis pathway has led to alternate reactor designs, specifically two-stage and plug flow digestion [31]. The two stage system typically features two continuous-flow reactors, one optimized for the hydrolytic and fermentative communities and the other for the methanogenic community. The organic input is continuously fed to the acetogenic reactor, with the high volatile fatty acid-containing output then transferred to the methanogenic reactor. This system enables maximum breakdown of the organic material as the acidified material can be loaded into

the methanogenic reactor in a controlled way such that the volatile fatty acid concentration and pH are optimized for methanogenic activity. Research evaluating two-stage systems have shown an increase in total energy production of 5-18% when compared to digestion using a single continuous flow system [31, 32].

The plug-flow reactor creates temporal separation between the fermentative and methanogenic stages similar to the batch digestion configuration, but also incorporates a continuous-flow component which allows for more flexibility during operation. While it often requires in higher hydraulic retention times compared to two stage digestion, total retention time is reduced compared to single tank batch digestion [28]. The microbial succession pattern in this reactor design follows a similar time-course to batch digestion, with identifiable hydrolytic, acetogenic, and methanogenic community profiles distinguishable in different sections of the digester. This configuration is likely more conducive to achieving maximum bacterial and archaeal productivity as the reactor design incorporates some level of physical distance and separation between the different metabolic functions in the methanogenesis pathway [17].

2.4. Production of Ethanol from sugar cane molasses

Molasses is the most commonly used raw material for production of ethanol. It contains about 50% of the total sugar; of which 30 to 33% is sucrose and rest is reducing sugar [33].

Molasses is obtained from the production of white sugar by repeated crystallization of sucrose in the mother liquor. It is the final molasses that obtained from the last massecuite, which is commonly used for the production of ethanol. The final cane molasses in Ethiopian sugar factories is with a purity of 34 to 40% which is a big loss if multiplied with the amount of molasses obtained from the sugar production which is in the range of 3 to 4% on cane [34]. These can be recovered by further crystallization by boiling of the final process. But, it is not economical to recover this loss with crystallization process. Therefore, ethanol production is used to recover this loss in the form of ethanol alcohol which is the best solution to the current shortage and price rise of energy.

Ethanol production from molasses consists of three major process steps. These are molasses treatment, fermentation, and distillation processes. In addition to theses main steps, evaporation for vinasses concentration is the very important plant which requires special attention.

Molasses Treatment: This stage is used for the reduction of the level of impurities in the molasses. Molasses treatment results in decreased level of inhibitory substances like Ca, Cu, and Fe in the molasses solution which improves ethanol production and calcium compounds which highly affect the efficiency of the plant by scale forming on the heating surface areas of the equipments in the later processes. The first process step in molasses treatment is dilution of the molasses from 80 – 86 ^{o}brix to 50 - 60^{o}brix to reduce its viscosity and therefore to facilitate heating. Then, the next step is heating the diluted molasses to temperature of 60 to 65oC which facilitates the reaction between calcium oxides and H_2SO_4. After preheating, its pH adjusted by addition of sulfuric acid (H_2SO_4) until pH becomes 4 to 5. Finally, the molasses heated to 95 to 100^{o}C in continuous operation.

Under the effect of both temperature and acidification, calcium sulfate ($CaSO_4$) precipitation, flocculation of long chain colloidal, and insolubilization of gums and waxes takes place within the molasses. Precipitated calcium sulfate is to be separated from the clear molasses by decantation processes

$$CaO + H_2SO_4 \longrightarrow CaSO_4 + H_2O$$

Fermentation: Fermentation is the breakdown or catabolism of organic compounds by microorganisms under both aerobic and anaerobic conditions. Various bacteria and yeasts metabolize sugars into ethanol through different pathways using different enzyme systems. Alcohol fermentation is used for the industrial production of alcohols and alcoholic beverages.

Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as fuel, is produced by fermentation processes. When saccharomyces species of yeast metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide as per the following reaction.

$$C_6H_{12}O_6 \longrightarrow C_2H_5OH + 2CO_2$$

Distillation: Distillation process of fermented beer is the next very important step in production of alcohol after fermentation. Distillation is a method of separating mixtures based on differences in volatility of components in boiling liquid mixture. It is a physical separation process. Once the alcohol is produced, it should be purified to the required quality. This is done by distillation

process. This step consumes a considerable amount of energy and is also a deciding factor in the quality and profitability of ethanol produced. Hence, in line with the demand of the industry, efforts have always been exerted to minimize requirement of energy and to improve the basic quality of alcohol produced. Ease of operation, reliability, lower down time and flexibility of operations are other parameters considered during the design of distillation columns.

Concentration of Vinasse

Due to large volume production (10 -15 liters per liter of ethanol), disposal of vinass is a headache for almost all ethanol plants. As a result, this liquor can therefore be processed into added-values animal feeds by concentration and, if necessary, drying and crystallization, precipitation of certain cations (e.g. potassium, sodium) [35]. In some ethanol plants, it also concentrated, mixed with filter cake from sugar factory and digested with bacteria. After full digestion, it is applied to cane field as fertilizer.

In MSF ethanol plant vinasse is being generated from primary column and is directed to quadruple effect falling film evaporators to reduce its water quantity. After this stage there is not any treatment mechanism in place at MSF other than partially blending with filter cake. There is still surplus vinasse which is being discharged to water bodies and used as fertigation purpose.

Simplified Ethanol manufacturing process from Molasses is schematically shown in the following material flow chart.

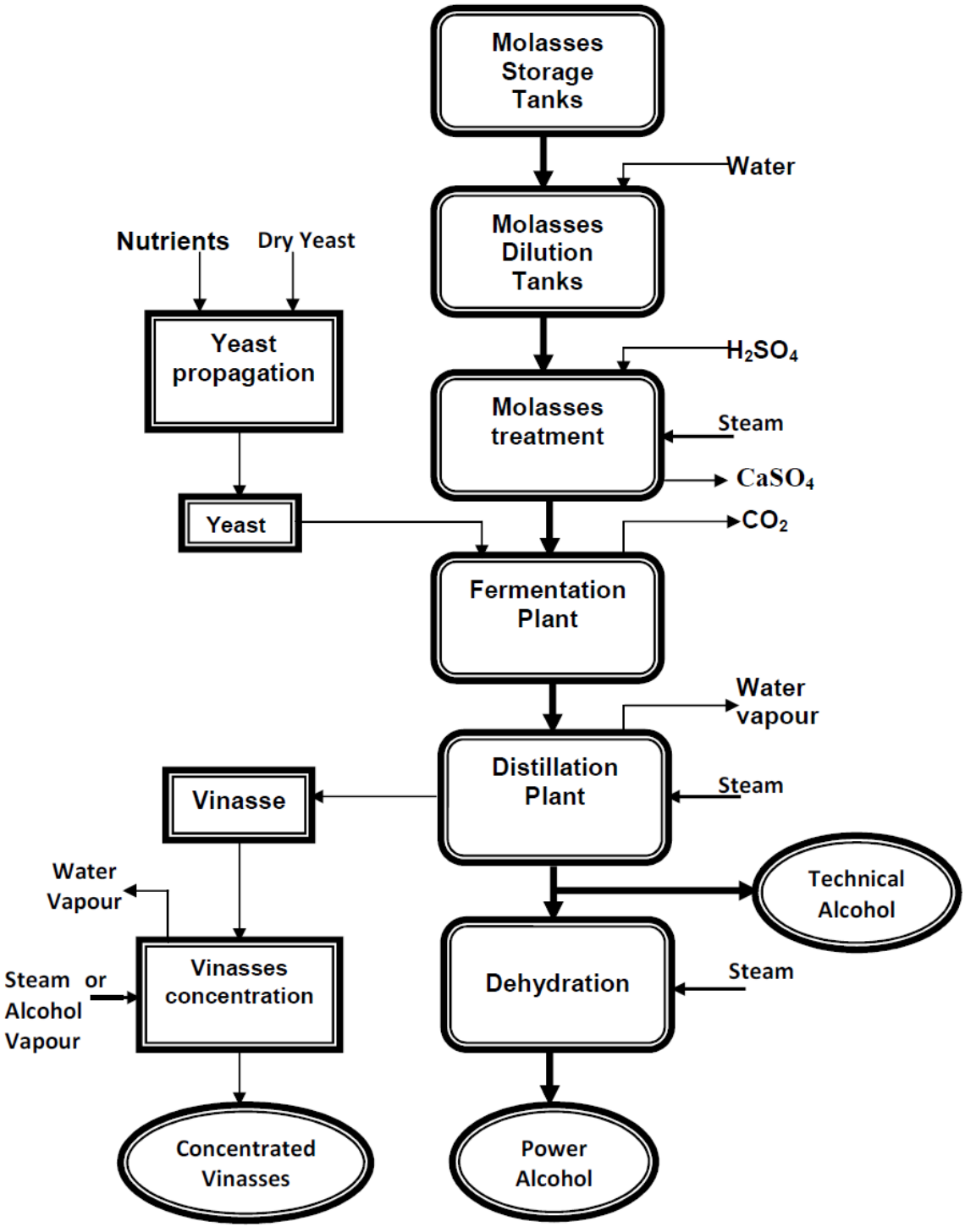


Figure 2.6: Process flow sheet of ethanol production from molasses

2.5. World Ethanol Production

Ethanol fuel production is increasing rapidly around the world. Ethanol with its high octane count is currently positively used as an automobile fuels with policies to promote its production most especially in Brazil, United States, majority of the European Union Countries and South Africa [38].

Table 2.2: Ethanol fuel production by country or region

World Fuel Ethanol Production by Country or Region (Million Gallons)									
Country	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
USA	6,521	9,309	10,938	13,298	13,948	13,300	13,300	14,300	14,806
Brazil	5,019	6,472	6,578	6,922	5,573	5,577	6,267	6,190	7,093
Europe	570	734	1,040	1,209	1,168	1,179	1,371	1,445	1,387
China	486	502	542	542	555	555	696	635	813
Canada	211	238	291	357	462	449	523	510	436
Rest of World	315	389	914	985	698	752	1,272	1,490	1,147
WORLD	**13,123**	**17,644**	**20,303**	**23,311**	**22,404**	**21,812**	**23,429**	**24,570**	**25,682**

(Data Source: Renewable Fuels Association.
http://www.ethanolrfa.org/resources/industry/statistics/#1454098996479-8715d404-e546)

Due to the ever increasing trend of world energy demand and environmental constraints, world has to search and deploy alternative renewable energy sources to avoid energy crisis. As a result, biofuels have drawn interest globally as supplement to oil based to serve as transportation or automobile fuels and also sources of heat and electricity generation supplies. Ethanol is the main biofuel which has been produced globally in large quantities. Ethanol as an alternative fuel, offers a Sustainable economy by reducing the use of imported petroleum, emitting neutral $CO_2(g)$, boost economy providing value added market opportunities for the Agricultural sector [38, 47].

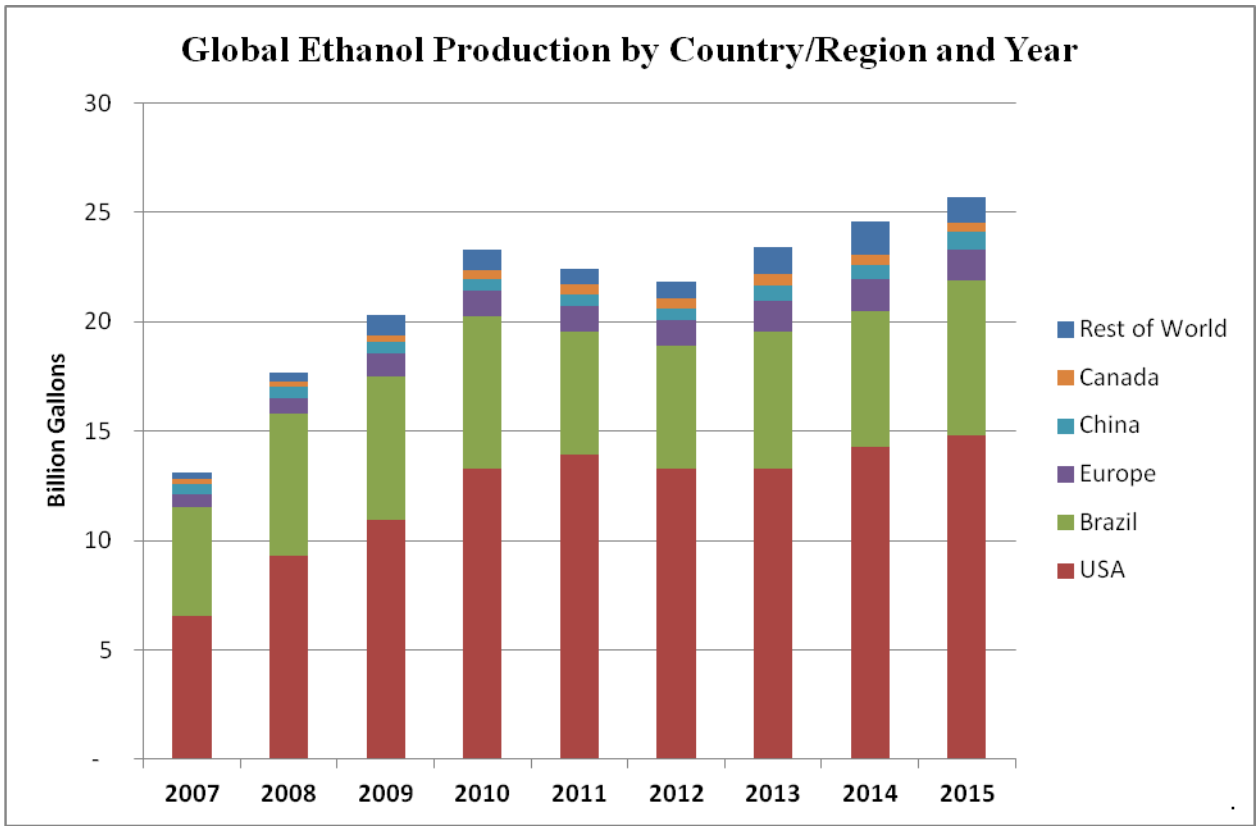


Figure 2.7: Global Ethanol Production by country/Region and year

(Data Source: Renewable Fuels Association.
http://www.ethanolrfa.org/resources/industry/statistics/#1454098996479-8715d404-e546)

2.6. Potential of Ethanol production in Ethiopia

In Ethiopia, it is only Metehara sugar factory and Fincha sugar factory that have their own ethanol manufacturing plant currently. However based on the data from Ethiopian sugar corporation, there are 10 sugar manufacturing project which will have their own integrated ethanol plant. Envisaged ethanol production upcoming projects are expected to be 230,168 million liters per year [13]

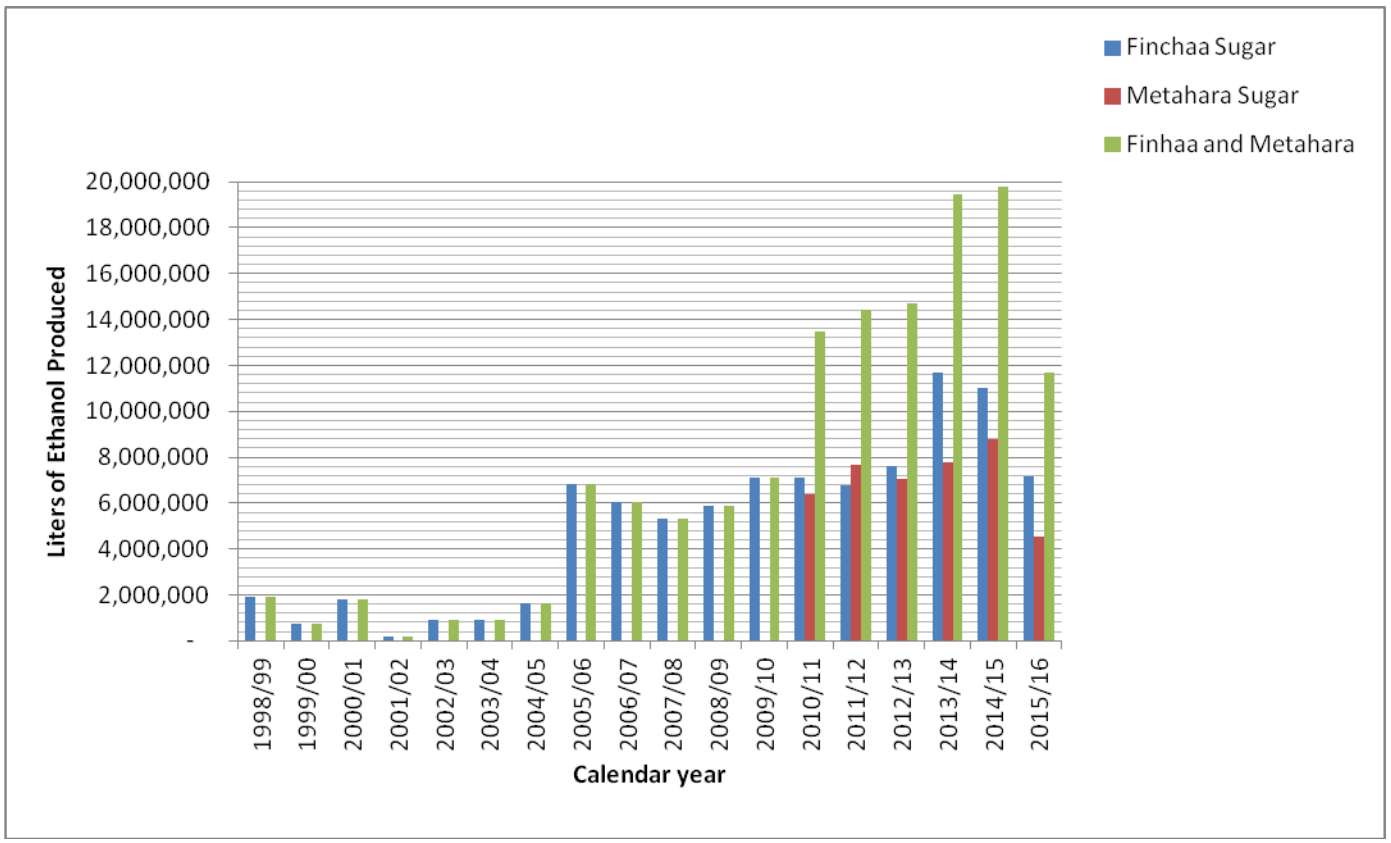


Figure 2.8: Current ethanol production in Ethiopia (Ethiopian Sugar Corporation)

By 2009 annual ethanol production was 7 million 117 thousand liters. In 2015 it has increased to 19 million 804 thousand liters a year. 18 million 480 thousand liters is used as fuel blended with benzene [13] (Table 2.3). Due to technical problems in the factories there is not consistency in the production figure.

The following table (2.3) shows future sugar and Ethanol production in Ethiopia.

Table 2.3: Future ethanol and sugar production in Ethiopia

(Source: http://ethiopiansugar.com/index.php/en/factories)

Sr.No	Factory Name	Sugar Production Per year (Ton)	Ethanol Production per year (M^3)
1	Metehara Sugar Factory	136,692	12,500
2	Finchaa Sugar Factory	270,000	20,000
3	Wonji Sugar Factory	220,700	12,800
4	Kesem Sugar Factory	260,000	30,000
5	Tendaho Sugar Factory	300,000	31,000
6	Omo - Kuraz project 1	556,000	52,324
7	Omo - Kuraz project 2	278,000	26,112
8	Omo - Kuraz project 3	278,000	26,112
9	Omo - Kuraz project 4	278,000	26,112
	Tana Beles Project 1	242,000	20,827
10	Tana Beles Project 2	242,000	20,827
11	Wolkait Project	484,000	41,654
	Grand total	**3,545,392**	**320,268**

Assuming average of 12 liters of vinasse per liter of ethanol, Ethiopia will be producing 3.8 billion liters of vinasse per year.

Table 2.4 shows projected Vinasse generation from ethanol production process based on cane sugar Molasses, assuming 12 liters of vinasse per liter of ethanol.

Table 2.4: Projected generation of Vinasse from each factory

Sr.No	Factory Name	Ethanol Production per year (M^3)	estimated Vinasse generation (M^3)
1	Metehara Sugar Factory	12,500	150,000
2	Finchaa Sugar Factory	20,000	240,000
3	Wonji Sugar Factory	12,800	153,600
4	Kesem Sugar Factory	30,000	360,000
5	Tendaho Sugar Factory	31,000	372,000
6	Omo - Kuraz project 1	52,324	627,888

7	Omo - Kuraz project 2	26,112	313,344
8	Omo - Kuraz project 3	26,112	313,344
9	Omo - Kuraz project 4	26,112	313,344
10	Tana Beles Project 1	20,827	249,924
11	Tana Beles Project 2	20,827	249,924
12	Wolkait Project	41,654	499,848
	Grand total	**320,268**	**3,843,216**

2.7. Characteristics of Sugarcane Vinasse

The characteristics of vinasse depend mainly on the raw material used for bio-ethanol production. Maize, barley and wheat have a high proportion of insoluble solids, which are separated by centrifugation and mixed with syrup obtained from the soluble solids while vinasse with high concentrations of soluble solids can be obtained when sugar cane, sugar beet, grape, agaves or sweet sorghum are used [72]. From these feed stocks, 9 to 14 L of wastewater can be obtained per liter of alcohol [72]. In Ethiopia, sugar cane is the only feed stock used for the production of ethanol and vinasse is generated from ethanol production from sugar cane molasses. Sugar cane molasses is a product of the concentration of juice and the precipitation of sugar and some non-sugar impurities present in the juice which are separated by the addition of chemical reactants such as SO_2, $Ca(OH)_2$, and phosphoric acid [73]. Due to the crystallization process, molasses has higher concentrations of potassium, phosphates, sulfates, calcium, iron, sodium, chlorides, carbon source and other trace elements than sugar cane juice [43].

Information available in the literature suggests that the major organic components of sugar cane vinasse are glycerol, lactic acid, ethanol and acetic acid. The main organic acids found are oxalate, lactate, acetate and malate and other alcoholic compounds, carbohydrates and a high content of phenols [74]. Composition and yield of vinasse from different raw materials is indicated in the appendix.

To characterize distillery wastewater in detail so that proper insight may be gained in attempt to treat the waste to reduce the pollution hazards, oxygen consumption values can use to quantify the amount of organic matter present in wastewater. However, considerable work has been reported in this field and should be taken into account with the characteristics of distillery

wastewater. Some of the work done on distillery waste characterization by various parameters like: - pH, COD, BOD, phosphate, total solids, total dissolved solids, total suspended solid, ammonia, sulfate, color and iron etc were clearly indicated in Table 2.4 [36, 37].

Table 2.5: Typical characteristics of distillery spent wash

Parameter	Range
pH	3.8-4.4
Total solids (mg/l)	60000 – 90000
Total suspended Solids (mg/l)	2000 – 14000
Total volatile solids (mg/l)	45000 – 65000
Total dissolved solids (mg/l)	58000 – 76000
COD (mg/l)	70000 – 98000
BOD_5 (mg/l)	45000 – 60000
Total nitrogen as N (mg/l)	1000 – 1200
Potash as K2O (mg/l)	5000 – 12000
Phosphate as PO4 (mg/l)	500 – 1500
Acidity as CaCO3 (mg/l)	8000 – 16000
Temperature (after heat exchanger) ^oC	70 – 80

2.8. Main Environmental Impacts of Vinasse

Distillery wastewater is usually comprised of a high volume of greatly acidic matter which presents many disposal and treatment problems. Waste streams generally contain high levels of both dissolved organic and inorganic materials. There has been increasing interest in the use of ethanol from biomass as a liquid fuel alternative [43,72]. Ethanol fermentation is examined in relation to distillery wastes. Reducing the volume of wastewater may be accomplished by fermenting higher strengths of molasses.

Following are the impacts of vinasse on environment [1, 3, 4, 72].

- Discharge of wastewater with high TDS would have adverse impact on aquatic life and to make unsuitable water for drinking purpose, if used for irrigation reduce the crop yield ,corrosion in water system and pipe line.

- Suspended solids in wastewater reduce the light penetration and plant production as a result in receiving water by increasing turbidity it can also clog the fish gills.
- High amount of BOD in the wastewater leads to the decomposition of organic matter under the anaerobic condition that produces highly objectionable products including Methane (CH4), Ammonia (NH3), and Hydrogen Sulphide (H2S) gas.
- Low Dissolved Oxygen (DO) in water bodies affect the aquatic life as DO drops fish and other species are threatened and may get killed.
- Fall in DO levels causes undesirable odors, tastes and reduce the acceptability of water for domestic purpose.
- In steam generation, DO is one of the most important factors causing corrosion of the boiler material.
- Generally, industrial wastewater changes pH level of the receiving water body. Such changes can affect ecological aquatic system; excessive acidity particularly can result in release of hydrogen sulphide (H_2S) to air.
- Alkaline nature of wastewater causes declination in plant growth and crop growth.
- Vinasse is red brown in color with unpleasant odor.

Due to the problems discussed above, the anaerobic treatment is more adequate for this type of waste compared to other treatment alternatives. In the literature, good results for the COD reduction have been reported (up 95.9%) [5]. Theoretical data have been used to estimate the potential of biogas from anaerobic digestion of vinasse, which is to 14.6m^3 per 1m^3 of vinasse. [5]

Zero Discharge of Distillery Wastewater

Worldwide environment regulatory authorities are setting for discharge of wastewaters from industries. In India for instance, distillery industry had been told to achieve zero discharge of vinasse/spent wash by December 2005 according to the Central Pollution Control Board as in the Figure 2.7 [36]

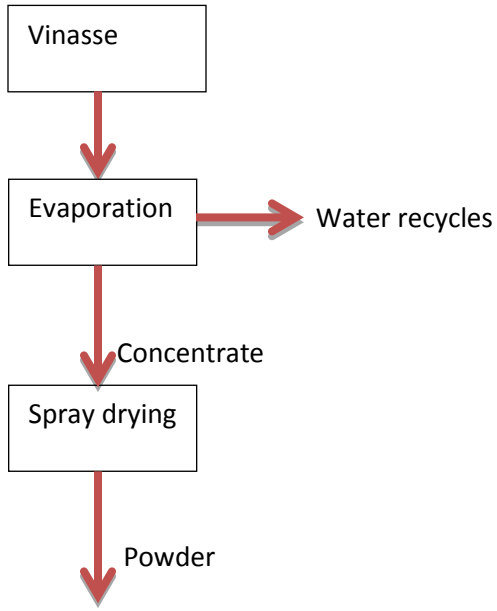


Figure 2.9: Zero Liquid discharge Policy

2.9. Anaerobic digestion of vinasse

Distilleries are producing large amount of vinasse (waste water from the production of ethyl alcohol by fermentation), which is characterized by low pH; high BOD and COD. The production of one liter of ethanol generate on average between 10-15 liters of vinasse. Due to its large volume of generation from distilleries utilization of vinasse is very essential and mandatory and attempts have been made all over the world to solve the problem [2, 3, 5].

Vinasse can be effectively purified by anaerobic digestion. The process produces low sludge, thereby facilitating its removal. Moreover, vinasse is chemically very complex and is rich in minerals such as potassium, calcium, and sulfur, and has a high content of organic matter, characterized by high levels of BOD and COD. Despite its strengths, it should not be forgotten that the vinasse is important source of pollution when discharged without precautions [1, 3, 5]

Vinasse typically contains adequate amounts of nutrients (both inorganic and organic) to support biological treatment for the removal of carbonaceous BOD. In Brazil, most of the vinasse that results from ethanol production is being used as fertilizer due to high potassium content [36].

In anaerobic degradation of vinasse, microorganisms are used to degrade the organic matter in the fluid in the absence of oxygen. Bacteria, rotifers, and protozoa are the main microorganisms used by this method [41]. After digestion, the following are produced: a clearer liquid, sludge, and methane gas. The anaerobic digestion encompasses the following stages as discussed in section 2.1.

The following chemical reaction represents hydrolysis of simple sugar glucose, as an example [41]

$$2C_6H_{12}O_6 + H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

With the formation of acetic acid, carbon dioxide and hydrogen, methane is, then, formed by the two following pathways:

$$2CH_3COOH \longrightarrow 2\,CH_4 + 2\,CO_2$$
$$4H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O$$

Satyawali et al. (2007) (as cited by Lucina Márcia, 2013), have reviewed the existing status and advances of various treatment methods. The authors have stated that anaerobic treatment was the most attractive primary treatment due to the BOD removal rate being over 80%, in addition to the energy recovery in the form of biogas. [41]

Ribas (2006) (as quoted by Kuusito, 2013 [41], has stated that the anaerobic reactors have shown to be a promising alternative because they accomplish a high rate of organic load removal and produce biogas. Additionally, this type of treatment has already been tested and used in many countries to treat the effluent from alcohol industries [41].

Anaerobic digestion is advantageous due to it efficient reduction of organic load and generation of biogas as a source of energy [43]. Ahring et al. (1994) concluded that the organic load introduced to a thermophilic anaerobic reactor may be above 30 kg COD/m^3-day [44]. Samuel et al. (2011) concluded that due to the increasing rate of vinasse disposal in Brazil, the better option is to promote the anaerobic digestion of the vinasse and produce biofertilizer and biogas.

Anaerobic digestion is successfully implemented worldwide on full scale by over 147, and anaerobic digestion of vinasse presents a sustainable and economically viable method allowing

mitigating the environmental impacts of ethanol industry [46]. However, most the studies have difficulties to describe the optimized parameters for production of biogas from vinasse.

Driessen et al. (1994) as cited by Kuusito [41] conducted a study on the vinasse digestion using UASB, with data collected from representatives in Brazil, India, Venezuela and the Netherlands. They showed the importance of the correct choice of parameters for each type of treated effluent for different geographic locations. The rate of COD removal varied between 65 and 95%, with feeding rates up to 22 kg/m3 day. In 1981, the IPT (Institute for Technological Research of São Paulo, Brazil) conducted an experiment in Penedo Agro Distillery (PAISA), in Penedo, Brazil, which investigated the anaerobic digestion of vinasse at 32° C, utilizing two UASB reactors with 11 and 24 m^3. The results included an average biogas production of 13.1 liters per liter of vinasse, with 65% CH_4 [41].

2.10. Biogas Production Technologies from Organic Wastes

Bacteria degradation of biological and organic matter in the absence of oxygen known as Anaerobic Digestion generates Biogas. The Anaerobic digestion is an effective proven technology for handling and treating biological wastes and effluents for generation of district heating and electricity supplies, as well as clean environment. Depending on the feedstock, Biogas is principally mixture of methane (CH_4)g, Carbon dioxide (CO_2)g and minute traces of hydrogen sulphide (H_2S)g, hydrogen, nitrogen, ammonia (NH_3)g and sulfur dioxide (SO_2)g. Methane is the only constituent of Biogas with significant fuel value. The inert diluents of Carbon dioxide (CO_2)g and nitrogen lowers the calorific content of the gas, while hydrogen sulphide (H_2S)g, corrosive nature wears down the anaerobic digester and pipes involved in the gas distribution [38].

In recent years energy considerations and environmental concerns have further increased the interest in direct anaerobic treatment of organic industrial wastes and the management of organic solid wastes from industry is increasingly controlled by environmental legislations [40]. Industries using AD for wastewater treatment range from: [40]

- Food processes: e.g. vegetable canning, milk and cheese manufacture, slaughterhouses, potato processing industry
- Beverage industry: e.g. breweries, soft drinks, distilleries, coffee, fruit juices

- Industrial products: e.g. paper and board, rubber, chemicals, starch, pharmaceuticals

Industrial biogas plants bring about a number of benefits for the society and the industries involved:

- Added value through nutrient recycling and cost reductions for disposal
- Utilization of biogas to generate process energy
- Improved environmental image of the industries concerned, through environmental friendly treatment of the produced wastes

Methane potential fraction differs and ranges between 40%-80% do the basis of the digester type, substrate quality and digesting bacteria [39].

2.11. Biogas Technologies and Trends of Application in Ethiopia

In Ethiopia Biogas production has been limited to hose hold applications for the last four decades. Based on the feasibility report prepared by SNV Biogas technology was introduced in Ethiopia as early as 1979, when the first batch type digester was constructed at the Ambo Agricultural College [48, 50]. Among 1000 biogas plants constructed, only 50 % are operational due to a lack of effective management and follow-up, technical problems such as lack of optimized parameters with the available feedstock, loss of interest due to either low or no production of methane gas, reduced animal holdings, evacuation of ownership, and water [48].

As only 2 % of Ethiopia's rural households have access to the national grid and 85 % of the population live and work in rural areas, the lack of energy severely restricts Ethiopia's social, environmental and economic development [48]. Woody biomass represents the principal form of cooking and lighting fuel in Ethiopia's rural areas. Such trends was caused deforestation and land degradation. Thus, well optimized biogas plant may offer an attractive option to replace unsustainable utilization of wood and charcoal. Based on the above facts, currently biogas is part of Ethiopia's Energy Policy and Environmental Protection Strategy, and also aligns with the Climate Resilient Green Economy (CRGE) strategy of the country. Biogas is a renewable resource that addresses the basic needs of rural households amongst which energy is one of it. Furthermore, its by-product – bio-slurry – enhances agricultural productivity and promotes organic farming, thus offering opportunities for niche markets and export [48, 49].

In 2007 a joint program between the Ethiopian Rural Energy Promotion and Development Centre (EREDPC) and SNV/Ethiopia was established to assess the feasibility of implementing nationwide household biogas program [48, 50]. The National Biogas Program started with an

aim of a first – pilot – implementation phase with construction of 14,000 biogas plants and development of a commercially viable biogas sector. Up-scaling construction to 100,000 biogas plants is considered for a subsequent phase [48].

During the first phase of the project, about 8000 biogas were installed [49]. In the second phase (2014-2017) the program targets to install 20,000 plants. In 2014 alone, a total of 1,762 plants had been constructed summing up to 9,825 plants constructed since 2009 [49]. However, little has been done on the optimization of the parameters at pilot and large scale and on bioreactor design, such that the effectiveness and the contribution of these constructed plants on the national energy grid were not to the expected. Although activities are ongoing to construct additional house hold biogas plant by national biogas program of Ethiopia, little has been done to upgrade it to industrial scale and there is no visible movement to utilize agro industrial wastes for the production of biogas in Ethiopia.

Chapter 3: Materials and Methods

3.1. Materials and Reagents

The following equipments and materials were used during the laboratory work

- Chemicals such as hydrated ferrous sulphate (FeSO4.7H2O),, sodium hydroxide (NaOH), diluted nitric acid, H_2SO_4, HgSO4 , KOH, $K_2Cr_2O_7$, H_2SO_4, $HgSO_4$, Ag_2SO_4, Standard ferrous ammonium sulfate titrant (FAS), Potassium hydrogen phthalate standard, Sulfamic acid, Phosphate buffer solution, Magnesium sulfate solution, Calcium chloride solution, Ferric chloride solution, Sodium sulfite solution, Nitrification inhibitor, Ammonium chloride solution
- Buffer tablets, PH 4 and 8
- Modified plastic bottle of 3 liter were used for digestion process and pharmaceutical liquid glucose plastic holders were used to collect the generated gas
- Vinasse sample
- Inoculum
- Laboratory equipments such as water bath, Methane analyzer, gas tight syringes, pH meter, beakers different size and capacity, digital balance, spatula, filter paper, BOD incubator, incubation bottles, evaporating dishes, Muffle furnace for operation at 550°C, desiccator, drying oven, for operation at 103 to 105°C and 180 °C, magnetic stirrer, flasks and vacuum pump, buret, digestion vessel

3.2. Description of the sampling area

Metehara Sugar factory is located in Oromiya Regional State at 200 Kilo Meters from Addis Ababa to the East direction. It has currently 10,100 hectares of land covered with sugarcane. Until very recent times it was the best when it comes to its production capacity that is 136,692 tons of sugar a year. Through an expansion project conducted it came up with an ethanol producing plant by the end of 2010 [11]. Currently the factory's ethanol plant has a capacity of producing 12,500 Meter Cube ethanol per year and producing 90,000 m^3 of vinasse per year. The factory has 2460 permanent and 7540 seasonal employees. Total population supported in the sugar estate is around 66000.

The climate of Metehara sugar estate is tropical semi arid with seasonal wet and dry season. The mean maximum and minimum temperature are 32.76 and 17.5 ^{o}C respectively. Mean annual rain fall is 539.7 mm and its mean relative humidity is 57.69%. Its mean daily sunshine hour is 8.28. Wind speed measured at 2 m heights is 2.81m/s. mean daily pan evaporation is 6.8 mm/day.

3.3. Methods

3.3.1. Sample collection and preparation

Sample collection: Vinasse samples were collected from Metehara Sugar Factory Ethanol Plant. The vinasse samples were collected immediately after drainage from the ethanol factory pipelines (primary column) at interval of 8 hours in one day time. It was mixed and collected by using 20 liter plastic jerry can and transported to the laboratory of Environmental engineering chair of AAiT. The sample was stored at 4ºC prior to lab analysis.

Inoculum: For the digester startup, the inoculum was granulated sludge from the pilot scale anaerobic digestion of coffee husk collected from AAU College of natural science. The inoculum sludge was analyzed for TS, VS, and others at AAiT environmental engineering laboratory so that it had total (TS) and volatile (VS) solid concentrations of 5.51 and 32.3%, respectively (Table 3.1). The volume of the inoculum used was sufficient to occupy 20% of the volume of each reactor.

Analysis of Total solids, Volatile solids and Mineral solids were determined based on standard methods for determination of water and waste water [76].

Table 3.1: Characteristics of Coffee husk sludge for inoculums

Parameter	Value
Total Solids (%)	5.51
Volatile Solids (%)	32.3
Total Solids (mg/l)	60796
Volatile Solids (mg/l)	19423
Mineral Solids (mg/l)	41373

Sample preparation: As 7-9 % TS is very good for the performance of the anaerobic digestion of the process [14, 61], after the TS % of the collected vinasse sample were determined, which was 10.2%, appropriate amount of water was added to brought 7% TS. The amount of water

added for dilution to bring 10.2 % of TS to 7% of 1 liter substrate was calculated based on solids material balance. Accordingly, 1000 ml of vinasse and 460 ml of distilled water were combined for final solids concentration of 7 % TS. Prepared sample was transferred to digestion bottles based on the experimental design generated by design expert (table 3.2).

3.3.2. Experimental design

Using Design Expert 7.0.0 Surface response analysis central composite design, the laboratory experimental design of this work was determined. To identify optimum temperature and pH for high methane yield and low COD & BOD, the experiment work was conducted based on the following table 3.2.

Table 3.2: Experimental design from Design Expert 7.0.0 Surface Response Analysis

Run #	Factor 1 T (^{0}C)	Factor 2 pH	Response 1 COD (mg/l)	Response 2 BOD (mg/l)	Cummulative Biogas Yeild (ml/gCOD)	CH_4 yeild (ml/gCOD)
1	35	7.25				
2	35	7.25				
3	40	8				
4	35	7.25				
5	42.07	7.25				
6	40	6.5				
7	35	6.19				
8	27.93	7.25				
9	30	6.5				
10	35	7.25				
11	35	8.31				
12	30	8				
13	35	7.25				

3.3.3. Experimental set up

Thirteen plastic bottles with 3 liters size were used as the digester and inserted in a water bath (fig. 3.3). 20 % of the digesters volume was filled with inoculums to ensure healthy digester startup. The digesters were tightly locked using a cork and sealed by Teflon tape to avoid atmospheric oxygen leakage. The samples were operated at a temperature of 28, 30, 35, 40 and 42^{0}C and PH of 6.19, 6.5, 7.25, 8 and 8.3 based on design expert 7.0 response surface methodology central composite design. Generated biogas from each digester was collected and

measured. Temperature was adjusted using water bath. Sodium Hydroxide solution and nitric acid were used to adjust the PH of the sample.

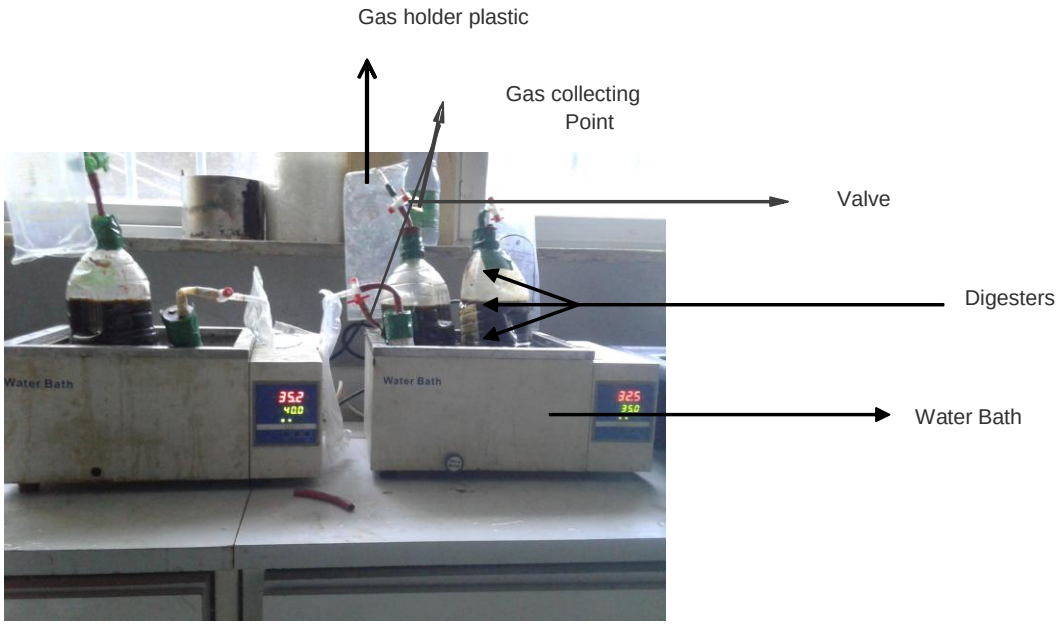


Figure 3.1: Experimental set up of the anaerobic digestion of vinasse

3.3.4. Experimental procedure

The bottles were filled with prepared substrate and the anaerobic digestion continued for hydraulic retention time (HRT) of twenty days inside the water bath at different mesophilic temperature stages. Shaking of digesters two times a day for 30 seconds was taking place manually to ensure proper mixing of the substrate. Digesters and gas collector were connected using flexible plastic hose so that the biogas could easily flow continuously and collected (Fig. 3.3). The collected gas was sucked each two days starting from the second day of digestion by using gas syringe and transferred to cylinder for measurement and characterization. Biogas volume was measured every two days by using graduated air locked gas syringe (Fig. 3.2). Gas measurement was started after two days of operation. Composition of biogas was measured using portable lab scale Geo tech gas analyzer model GA 5000 (Fig. 3.2) every two days interval

The slurry sample from the digester was collected at the end of the 20th day and its physicochemical parameters were tested using standard determination of methods of water and waste water discussed above section 3.3.5 below.

Figure 3.2: Geo tech gas analyzer model GA 5000

Figure 3.3: Gas syringe for measurement of biogas volume

3.3.5. Analytical Methods for characterization of vinasse before and after digestion

After vinasse samples were collected, the initial physicochemical characteristics of vinasse such as total solids TS (%), volatile solids VS (%), total dissolved solids (TDS), total suspended solids (TSS), Chemical Oxygen demand (COD), Biological Oxygen Demand, total nitrogen TN (%), total alkalinity, reactive phosphate and phenol were measured initially before exposed to anaerobic digestion. Additionally, after anaerobic digestion treatment parameters such as COD, BOD, pH, Biogas Volume and Methane composition were measured after 20 days hydraulic retention time (HRT). The experiment was conducted according to procedures given in standard methods for the examination of water and wastewater [76].

Total solids and volatile solids, Dissolved and suspended solids tests were performed at AAiT environmental engineering chair laboratory while the remaining parameters were conducted at Addis Ababa City Administration environmental protection laboratory.

3.3.6. Standard Method for determination of Solids

Determination of Total solids: Analysis of total solids was very important as its content has impact on the performance of anaerobic digestion and its subsequent methane production based on Flavia Liotta et.al, 2014 [51].

Total solids are the term applied to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids include "total suspended solids," the portion of total solids retained by a filter, and "total dissolved solids,'' the portion that passes through the filter.

A well-mixed 20 ml vinasse sample was pipeted to a pre weighted dish and evaporated and dried to constant weight in an oven at 103 to 105°C for 1 hour. The dish was cooled in desiccators to balance the temperature and weighed in digital balance (Appendix A1). The increase in weight of dried dish over that of the empty dish represents the total solids of vinasse and calculated based on equation 3.1 below.

Determination of Volatile Solids: The residue from total solids analysis was ignited to constant weight at 550°C for 1 hour. The ignited dish was cooled in desiccators to balance the temperature and weighed in digital balance (Appendix A2). The remaining solids represent the fixed total, dissolved, or suspended solids while the weight lost on ignition is the volatile solids and calculated based on equation 3.2.

The determination is useful in control of wastewater treatment plant operation because it offers a rough approximation of the amount of organic matter present in the solid fraction of wastewater, activated sludge, and industrial wastes.

Calculation

$$Total\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{…… ……3.1}$$

Where A – weight of dried residue + dish, mg, B – weight of dish, mg

$$Volatile\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{.....……3.2}$$

Where, A= weight of residue + dish before ignition, mg, B = weight of residue + dish or filter after ignition, mg.

Total Dissolved Solids

A well-mixed vinasse sample was filtered by using vacuum filter through a standard glass fiber filter and collected in a clean dish. The filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180°C for one hour. The sample was cooled in desiccators to balance the temperature and weighed in digital balance (Appendix A3). The increase in dish weight represents the total dissolved solids and calculated based on equation 3.3.

Calculation

$$Total\ Dissolved\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{…….. 3.3}$$

Where A – weight of dried residue + dish, mg, B – weight of dish, mg

Total Suspended Solids:

A well-mixed vinasse sample was filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103 to 105°C for 1 hour and cooled to balance the temperature (Appendix A4). The increase in weight of the filter represents the total suspended solids and calculated based on equation 3.4.

Calculation

$$Total\ Suspended\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{..... 3.4}$$

Where, A = weight of filter + dried residue, mg, and B = weight of filter, mg

3.3.7. Standard method for determination of BOD and COD

Determination of COD

Chemical oxygen demand (COD) is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. Because of its unique chemical properties, the dichromate ion (Cr_2O_7) is the specified oxidant used in this experiment.

COD measurements were carried out according to procedures given in standard methods for the examination of water and wastewater, titrimetric method [76].

50 ml of vinasse was oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acid solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced ($K_2Cr_2O_7$). is titrated with ferrous ammonium sulfate to determine the amount of ($K_2Cr_2O_7$).. By using ferroin indicator, the end point is a sharp color change from blue-green to reddish brown (Appendix A4). In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample consumed and the oxidizable matter is calculated in terms of oxygen equivalent based on equation 3.5.

Calculation

Chemical oxygen demand (COD) concentration was calculated using the following formula:

$$COD\ \left(\frac{mg}{l}\right) = \frac{(FASs - FASp)\times N\times f}{Vs} \quad \text{..... 3.5}$$

Where, FASs: used ferrous ammonium sulphate concentration for sample, mg/l, FASp: used ferrous ammonium sulphate concentration for pure water, mg/l, f: dilution factor,
N: normality of FAS and Vs: sample volume, ml

Determination of BOD

The biochemical oxygen demand (BOD) determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron.

Vinasse sample was filled to overflowing, an airtight bottle of the 100 ml and incubating it at 20^{o}C for 5 day (Appendix A5). Dissolved oxygen (DO) was measured initially and after incubation, and the BOD_5 was computed from the difference between initial and final DO based on equation 3.6.

Calculation

For each test bottle meeting the 2.0 mg/l minimum dissolved oxygen (DO) depletion and the 1.0 mg/l residual DO, calculate BOD5 as follows:

$$BOD5\left(\frac{mg}{l}\right) = \frac{D1 - D2}{P} \quad \text{..... 3.6}$$

Where;

D1 = DO of diluted sample immediately after preparation, mg/l,

D2 = DO of diluted sample after 5 day incubation at 20 °C, mg/l,

P = Decimal volumetric fraction of sample used,

3.4. Kinetic Model of Biogas Production

Biogas production kinetic was modeled through modified Gompertz equation [57]. Kinetic of biogas production in batch anaerobic digestion process is proportional to specific growth rate of methanogenic bacteria in digester [54-56] based on the following equation.

$$y(t) = ym \times \exp\left\{-exp\left[\frac{U \times e}{ym}(\lambda - t) + 1\right]\right\} \quad \text{..... 3.7}$$

Where,

y(t) = The cumulative biogas yield at a digestion time t days (mL/g COD)

ym = The biogas production potential (mL/g COD)

U = The maximum biogas production rate (mL/g COD.day)

λ = Lag phase period or minimum time to produce biogas (days)

t = Cumulative time for biogas production (days)

e = Mathematical constant (2.718282)

Kinetic constant of y_m, U, and λ was determined using non-linear regression with help of polymath software [54-56].

3.5. Design of Up - Flow Anaerobic Sludge Blanket (UASB) reactor

A mass balance for the mass microorganisms in a complete mix reactor shown in fig 3.4, below is given by equations based on Metcalf and Eddy [6].

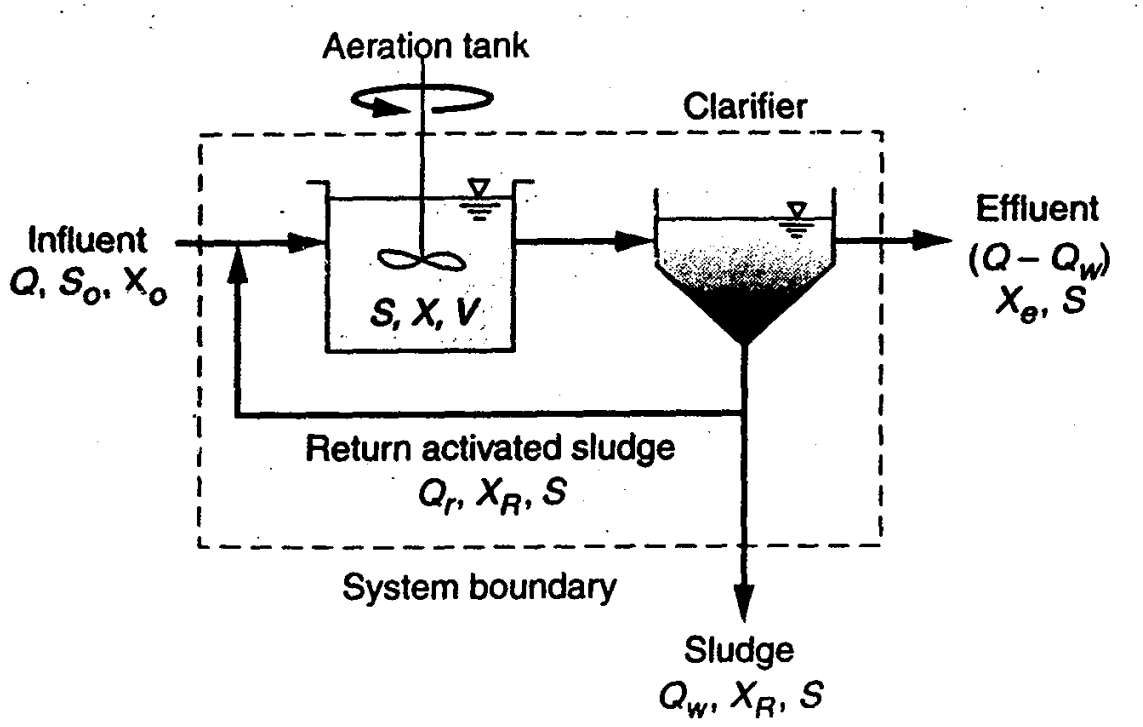


Figure 3.4: Schematic diagram of activated sludge process

Accumulation = Inflow – Outflow + Net growth

$$\frac{dX}{dt}V = QX_o - [(Q - Q_w)X_e - Q_w X_R] + r_g V \quad \ldots\ldots\ldots 3.8$$

Where,

dX/dt = rate of change of microorganism concentration in the reactor measured g VSS/m^3 day

V_r = reactor volume, m^3

Q = flowrate, m^3/day

Xo = concentration of biomass in influent, g VSS/ m^3

Q_w = waste sludge flowrate, m^3/day

X_e = concentration of biomass in the effluent, g VSS/m^3

X_r = concentration of biomass in return line, g VSS/m^3

$r_{g'}$ = net rate of microorganism growth, g VSS/m^3.day

Assume that the concentration of microorganisms in the influent is neglected and steady state condition prevail (dX/dt = 0), Equation 3.8 can be simplified to

$$(Q - Q_w)X_e + Q_w X_R = r_g V \quad \ldots\ldots\ldots 3.9$$

The substrate utilization rate in biological systems can be modeled with the following equation [6].

$$r_{su} = -\frac{kXS}{K_s + S} \quad \ldots\ldots\ldots 3.10$$

Where,

r_{su} = rate of substrate concentration change due to utilization, g/m^3.day

k = maximum specific substrate utilization rate, g substrate/g microorganism.day

X = biomass (microorganism concentration), g/m^3

S = growth limiting substrate concentration in solution, g/m^3

Biomass growth rate (r_g) is proportional to substrate utilization rate by the synthesis yeid coefficient, and biomass decay (k_d) is proportional to the biomass present based on Metcalf and eddy [6].

Thus,

$$r_g = -Yr_{su} - k_d X \qquad \text{……..3.11}$$

Where,

$r_{g'}$ = net rate of microorganism growth, g VSS/m^3.day

Y = synthesis yield coefficient, g VSS/g COD

k_d = endogenous decay coefficient day^{-1}

X = biomass (microorganism concentration), g/m^3

Combining Eq. (3.9) with Eq. (3.10), yields Eq. (3.12)

$$\frac{(Q - Q_w)X_e + Q_w X_R}{VX} = -Y\frac{r_{su}}{X} - k_d \qquad \text{…….3.12}$$

By definition SRT (solids retention time) is the solids in the system divided by the mass of solids removed per day. Thus, Eq. (3.12) can be rewritten as

$$\frac{1}{\text{SRT}} = -Y\frac{r_{su}}{X} - k_d \qquad \text{……3.13}$$

Substituting Eq. (3.11) in to Eq. (3.13) yields

$$\frac{1}{\text{SRT}} = \frac{YkS}{K_s + S} - k_d \qquad \text{…….3.14}$$

The mass balance of substrate utilization in aeration tank (see Fig.3.4) is

Accumulation = Inflow – Outflow + Net growth

$$\frac{dS}{dt}V = QS_o - QS + r_{su}V \qquad \text{……3.15}$$

Where,

So = influent substrate concentration, g/m^3

S = efffluent substrate concentration, g/m^3

r_{su} = rate of substrate concentration change due to utilization, g/m^3day

Substituting the value of r_{su} (Eq. 3.11) in Eq. (3.10) and assuming steady state conditions (dS/dt = 0), Eq. (3.10) can be written as

$$S_o - S = \left(\frac{V}{Q}\right)\left(\frac{kXS}{K_s + S}\right) \quad \text{.....3.16}$$

If Eq. (3.14) is solved for the term (S/Ks+S), and substituted to Eq. (3.16), the following expression is obtained for biomass concentration in aeration tank

$$X = \left(\frac{\mathbf{SRT}}{\tau}\right)\left[\frac{Y(S_o - S)}{1 + (k_d)\mathbf{SRT}}\right] \quad \text{.....3.17}$$

The total mixed liquor volatile solids concentration (MLVSS) in aeration tank equals the biomass concentration X plus non biodegradable volatile suspended solids concentration (nbVSS), Xi

$$X_T = X + X_i \quad \text{.....3.18}$$

A material balance on inert material (nb VSS) is as follows

Accumulation = Inflow – Outflow + generation

$$(dX_i/dt)V = QX_{o,i} - X_iV/\mathrm{SRT} + r_{X,i}V \quad \text{.....3.19}$$

Where,

X_{oi} = nb VSS concentration in influent, g/m^3

X_i = nb VSS concentration in aeration tank, g/m^3

r_{xi} = rate of nb VSS production from cell debris g/m^3.day

The rate of production of cell debris is proportional to the endogenous decay rate

$$r_{Xd} = f_d(k_d)X \quad \text{.....3.20}$$

Where,

r_{xd} = rate of cell debris production, g VSS/m^3.day

f_d = fraction of biomass that remains as cell debris, 0.1 – 0.15 g VSS/g VSS

At steady state (dXi/dt = 0) and substituting Eq. (3.20) for r_{xi} in Eq. (3.19) yields

$$X_i = X_{o,i}\,(\mathrm{SRT})/\tau + (f_d)(k_d)X(\mathrm{SRT}) \qquad \text{.....3.21}$$

Combining Eq. (3.17) and Eq. (7.21) for X and Xi and substituting to Eq. (3.18) yields

$$X_T = \left(\frac{\mathrm{SRT}}{\tau}\right)\left[\frac{Y(S_o - S)}{1 + (k_d)\mathrm{SRT}}\right] + (f_d)(k_d)(X)\mathrm{SRT} + \frac{(X_{o,i})\mathrm{SRT}}{\tau} \qquad \text{.....3.22}$$

Solids Production from biological reactor represents the mass of material that must be removed each day to maintain the process and calculated by the following formula [6].

$$P_{X_T,\mathrm{VSS}} = \frac{X_T V}{\mathrm{SRT}} \qquad \text{.....3.23}$$

Where,

$P_{X,T}$, VSS = total solids wasted daily, g VSS/day

X_T = total MLVSS concentration in aeration tank g VSS/m3

SRT = Solids retention time, day

By substituting Eq. (3.22) for XT in Eq. (3.23) and replacing τ (hydraulic retention time) with V/Q, the amount of VSS produced and wasted daily can be determined as follows.

$$P_{X,\mathrm{VSS}} = \frac{QY(S_o - S)}{1 + (k_d)\mathrm{SRT}} + \frac{(f_d)(k_d)YQ(S_o - S)\mathrm{SRT}}{1 + (k_d)\mathrm{SRT}} + Q\,X_{o,i} \qquad \text{.....3.24}$$

Where:

$P_{X,T}$, VSS = net mass of cell tissue produced per day, g VSS/day

Q = flow rate, m^3/day

X_{oi} = nb VSS concentration in influent, g/m^3

k_d = decay coefficient g VSS/g COD.day

So = COD in influent, mg/l

S = COD in effluent, mg/l

Y= yield coefficient, g VSS/g COD

f_d = fraction of biomass that remains as cell debris g VSS/g VSS

k_d = endogenous coefficient, day^{-1}

SRT = Solids resident time, day

Volume of Methane gas generated from anaerobic digestion can be calculated by using the following equation [12].

$$V_{CH4} = 0.35\left[(So - S) \times \frac{(Q)1kg}{1000g} \times -1.42P_x\right] \quad \text{.....3.25}$$

Where, VCH_4 = volume of methane produced at standard conditions (0ºC and 1 atm), m^3/day;

0.35 = theoretical conversion factor for the amount of methane produced from the complete conversion of one gram of COD to methane and carbon dioxide, ml CH_4/g COD oxidized

Q = flow rate, m^3/day

So = COD in influent, mg/l

S = COD in effluent, mg/l

P_x = net mass of cell tissue produced per day.

The volume of the digester was calculated based on the equation 3.11 [6]

$$V_l = \frac{Vn}{E} \quad \text{..... 3.26}$$

Where, V_l – total reactor volume

E – Effectiveness factor

$$V_n = \frac{Q \times S_o}{L_{org}} \quad \text{..... 3.27}$$

Where, Vn = effective liquid volume of reactor, m^3

Q = influent flowrate, m^3/day

So = influent COD, kg COD/m^3

Lorg = organic loading rate, kg COD/m^3.d

3.6. Expected methane gas production

A COD balance can be used to a count for the changes in COD during fermentation. COD loss in anaerobic reactor is accounted for methane production. Based on the stoichiometry of the following reaction, the COD equivalent of Methane can be determined.

$CH_4+2O_2 \longrightarrow CO_2+ 2H_2O$

The COD of methane is the amount of oxygen needed to oxidize methane CO_2 and H_2O. From the above reaction one mole of methane requires 2 moles of oxygen to convert it CO_2 and H_2O. COD per mole of methane is 2(32 g O2/mole) = 64 g O2 per mole. The volume of methane per mole at standard conditions (0^oC and 1 atm) is 22.414L.

$$CH4\ equivalent\ of\ COD\ converted = \frac{22.414\ l}{64\ g} = 0.35l\frac{CH4}{g.COD} \quad \text{…….....} \quad 3.28$$

The volume of gas occupied by 1 mole of gas at a given temperature is determined by universal gas law

$$V = \frac{n \times R \times T}{p} \quad \text{…..……} \quad 3.29$$

Where,

V = volume of a gas occupied by 1 mole of gas at a give temperature, l

R = universal gas constant, 0.082057 atm.l/mole.K,

T = temperature, K (273.15+0C)

P = absolute pressure, atm

3.7. Statistical Analysis

3.9.1. Response Surface Methodology:

RSM is a collection of mathematical and statistical techniques for empirical model building. By careful design of experiments, the objective is to optimize a response (output variable) which is influenced several independent variables (input variables). To facilitate the combined effect of environmental factors; pH and temperature, the statistical experiments was designed by response

surface methodology (RSM). An improvement in product yield, a reduction in process variability, a closer confirmation of the output response and a reduction in the experimental time and overall costs are the outcomes of using this statistical approach [58].

In this thesis, RSM with a central composite design (CCD) was used to optimize the process parameters affecting COD, BOD and methane yield from vinasse. The individual and interactive effects of pH and mesophilic temperature on methane yield, effluent COD and effluent BOD were investigated. Significance and adequacy of models was studied by analysis of variance (ANOVA). The statistical analysis of the experiments was conducted by design expert 7.00.

3.9.2. Non linear regression

Non linear regression is a form of regression analysis in which observational data are modeled by a function in which a non linear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximations. The constants from modified Gompertz equation ym (biogas production potential (mL/g COD), U (the maximum biogas production rate, mL/g COD.day) and λ (lag phase period or minimum time to produce biogas, days) were determined using non-linear regression with help of polymath 5.1 software.

Chapter 4: Results and discussion

4.1. Characterization of Initial Vinasse

The physicochemical characteristics of initial vinasse collected from Metehara Sugar Factory (MSF) distillery plant were indicated in the table 4.1 below.

The concentration of COD and BOD of vinasse sample collected from MSF were very high compared to the standard (Table 4.1). Industrial waste waters with much higher biodegradable COD concentrations were suitable for anaerobic treatment [6]. Thus COD of vinasse used as a substrate in this thesis work was ideal for biogas generation. The COD/N ratio of this vinasse is 489/7 which in the range of optimum COD/N value reported by Speece, 1996 [66].

Table 4.1: The physic chemical characteristics of vinasse collected from MSF

Parameter	Value
Total Solids, mg/l	103750
Volatile Solids, mg/l	39375
pH	4.15
Total suspended solids, mg/l	65520
Total dissolved solids, mg/l	38230
Mineral Solids, mg/l	64375
Chemical oxygen demand, mg/l	51987
Biological oxygen demand, mg/l	30581
Total Nitrogen, mg/l	750
Total alkalinity, mg/l	13950
Reactive phosphate, mg/l	1665
Phenol, mg/l	24

The high concentration of total solid in MSF vinasse (103750 mg/l) was also leads to total biogas production. Similarly, Budiyono *et al.2014* [63] described that the more total solid value of vinasse, the more organic matter contained in vinasse that will be ready for digestion. In the present study, although the TS concentration was very high which was beyond the optimum range for biogas digestion, using de-mineralized water the total solid concentration was diluted and reduced to 7.015-9.310% TS which was the optimum range for anaerobic fermentation. Similar to the present study results, Budiyono *et al.2010* [61, 71] also reported the same result

that solid concentration of 7-9.2 % in substrates will generated biogas optimally. During fermentation of vinasse in the digester, pH is function of time [56]. In the present study, initial pH of 7 gave better result than the others (pH of 6 and 8). In supporting to the present result, Budiyono *et al.* [67] stated that pH of 7 is good condition for anaerobic bacteria to adapt in digester.

Vinasse contains phenolic compounds in large amount, which is 61-469 mg/L [56]. Phenolic compounds have anti-microbial characteristic. Presence of phenolic compounds in vinasse disrupts degradation process of organic materials in anaerobic digester [64]. The phenolic content of this vinasse lower than the values indicated in literature [56]. This might be due to the dilution of the TS with de-mineralized water.

The alkalinity of the MSF vinasse was higher and it will help to keep the effluent pH at or neutral point. This level of alkalinity based on Metcalf and eddy, 2003 [6] avoids the requirement to purchase chemicals for pH control that could have significant impact on the economics of anaerobic treatment. In addition the amount of volatile solids (39375 mg/l) shows the availability of enough organic matter in the substrate that could be used as a food for microorganisms for subsequent decomposition and production of biogas.

4.2. Effect of Temperature and pH

Based on the experimental design seen in the table 3.2 section 3, digestion of vinasse was conducted at temperature of 28^{o}C, 30^{o}C, 35^{o}C, 40^{o}C, and 42^{o}C and a pH of 6.2, 6.5, 7.5, 8 and 8.3 for Twenty days HRT. At temperature of 35 ^{o}C and pH of 7.25, biogas and methane production were highest (34.68, ml/g COD and methane yield of 28.091 ml/ g COD) as well as COD and BOD removal efficiency was also better (64 and 76 % respectively) compared to the other variables (Table 4.2). Composition of biogas was 81 % CH_4, 14 % CO_2, 2 % O_2, 3 % other and 1 PPM H_2S.

The present study was also found a total amount of 8.297 liter of methane gas from a liter of vinasse, which is in the range of 5.11 – 15.03 reported by Baez-Smith (2006) [1]. At variables below and above this point, biogas production and COD & BOD removal efficiency was low.

This shows at around normal pH and mesohilic temperature (35 ^{o}C) anaerobic digestion showed more stability. This result is supported by Espinoza-Escalantea et.al 2009, [62] which stated that Mesophilic temperature (35^{o}C) and initial neutral pH has produced the most biogas yield.

RSM with Central Composite Design (CCD) was used to optimize methane yield, COD & BOD reduction from vinasse coming from fermentation and distillation of cane sugar molasses. The design matrix of the factors and experimental values of responses are tabulated in Table 4.2.

Table 4.2: Full factorial CCD matrix of Temperature and pH with response values of COD, BOD and Biogas and methane yield

Run	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4
	Temperature (^{o}C)	pH	COD (mg/l)	BOD (mg/l)	Biogas Yield (ml/g COD)	Methane Yield (ml/g COD)
1	35	7.25	18760	7645	34.68	28.0908
2	40	8	23677	10703	30.39	24.0081
3	42	7.25	26549	12232	27.51	20.6325
4	35	7.25	18720	7553	34.66	27.9013
5	40	6.5	35345	16054	21.97	13.8411
6	28	7.25	35544	16513	21.51	14.08905
7	35	7.25	18700	7339	34.6	28.0606
8	35	8.3	22424	9786	31.73	26.0186
10	30	6.5	35957	16819	21.06	12.7413
11	30	8	31372	14373	23.14	17.0079
12	35	7.25	18570	7339	34.3	27.783
13	35	7.25	18796	7798	34.7	28.0029

4.3. The effect of temperature and pH on COD reduction

The predicted values of COD were obtained from the quadratic model and by evaluating the relationship between pH and temperature. The statistical model was developed by applying multiple regression analysis using the experimental data of COD. The final equation in terms of actual factors is given below.

$$COD = 8.14028E^5 - 15298.1628 \times T - 1.36967E^5 \times pH - 472.2 \times T \times pH + 259.94166 \times T^2 + 10169.29034 \times pH^2$$

Table 4.3: ANOVA for Response Surface Quadratic Model

Source	Sum of squares	df	Mean Sqaure	F Value	p-value Prob > F	
Model	578226886.8	5	115645377	90.2455689	< 0.0001	Significant
A-Temperature	55271314.68	1	55271314.7	43.1317823	0.0006	
B-pH	98491052.28	1	98491052.3	76.8589393	0.0001	
AB	12542222.25	1	12542222.3	9.78750736	0.0204	
A^2	273931072	1	273931072	213.766136	< 0.0001	
B^2	137772561.5	1	137772562	107.512842	< 0.0001	
Residual	7688712.841	6	1281452.14			
Lack of Fit	7659020.041	2	3829510.02	515.883988	< 0.0001	Significant
Pure Error	29692.8	4	7423.2			

The Model F-value of 90.25 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This means both temperature and pH have individual effect on COD reduction as p value < 0.05. In addition the model terms AB indicates that pH and temperature have combined significant effect on COD reduction (p value <0.05). From the ANOVA of the model we can aslo see that the square of each factor (i.e. initial pH & Temperature) has the individual effect on the response (p value < 0.05).

The "Lack of Fit F-value" of 515.88 implies the Lack of Fit is significant. There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise.

Table 4.4: Post ANOVA analysis for COD

Std. Dev.	1132.012	R-Squared	0.986877
Mean	25367.83	Adj R-Squared	0.975942
C.V. %	4.462393	Pred R-Squared	0.834616
PRESS	96900927	Adeq Precision	21.99538

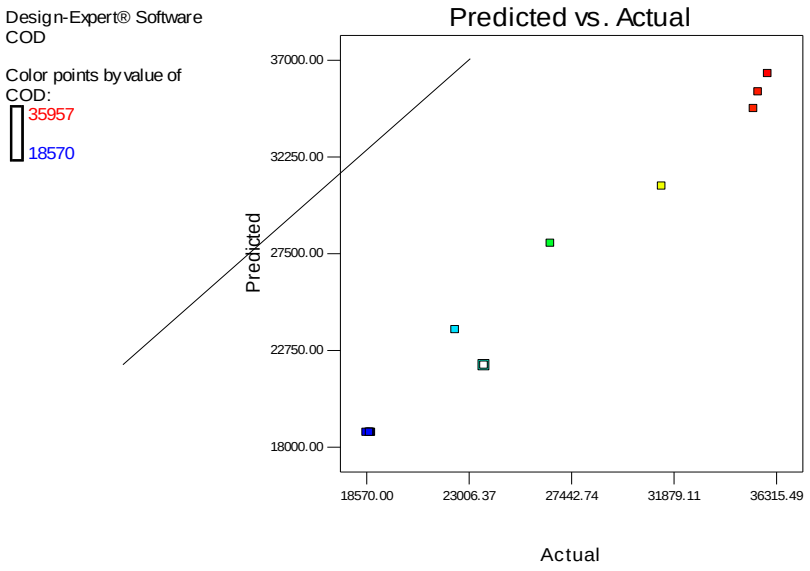
The "Pred R-Squared" of 0.8346 is in reasonable agreement with the "Adj R-Squared" of 0.9759. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of this experiment of 21.995 indicates an adequate signal. This model can be used to navigate the design space.

Table 4.5: ANOVA for COD standrd error and coefficient of estimate

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	18709.2	1	506.251349	17470.448	19947.952
A-Temperature	-2628.481374	1	400.226833	-3607.801	-1649.162
B-pH	-4529.786885	1	516.690619	-5794.083	-3265.491
AB	-1770.75	1	566.006215	-3155.717	-385.7827
A^2	6498.541395	1	444.474077	5410.9525	7586.1302
B^2	5720.225814	1	551.674617	4370.3267	7070.1249

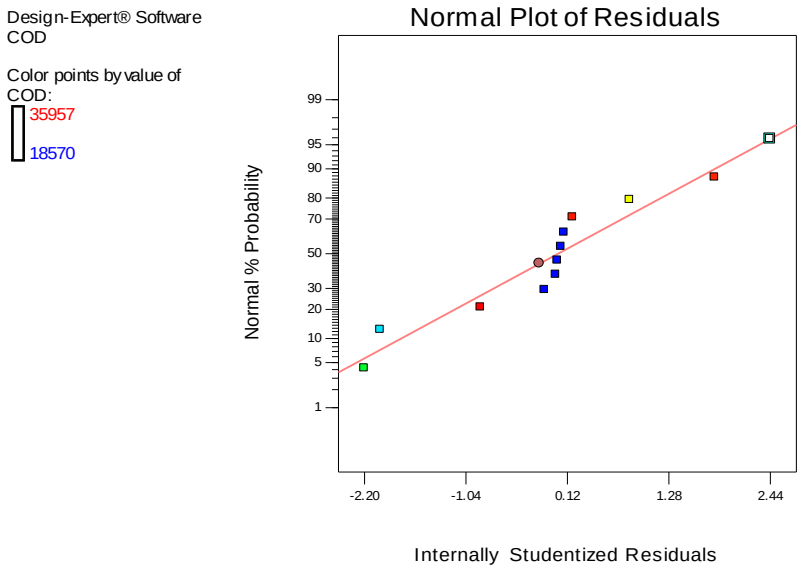
The R-squared of 0.986877 showed that the model could explain 98.68 % of the variability in the response . For a good statistical model, the R^2 should be in the range of 0.75–1.0 which indicates a good fit of the model [58]. The low value of coefficinet of varaition (4.462393) indicats that the good precision and reliabiity of the experiment.

Figure 4.1 (a-d) below shows the residual plotes of effluent COD. Normal probability plot shows that the residuals follow the normal distribition. The residuls vs predicted COD value follows random scatter which means the model exhibits constant varience, residuals vs run shows randomscatter.



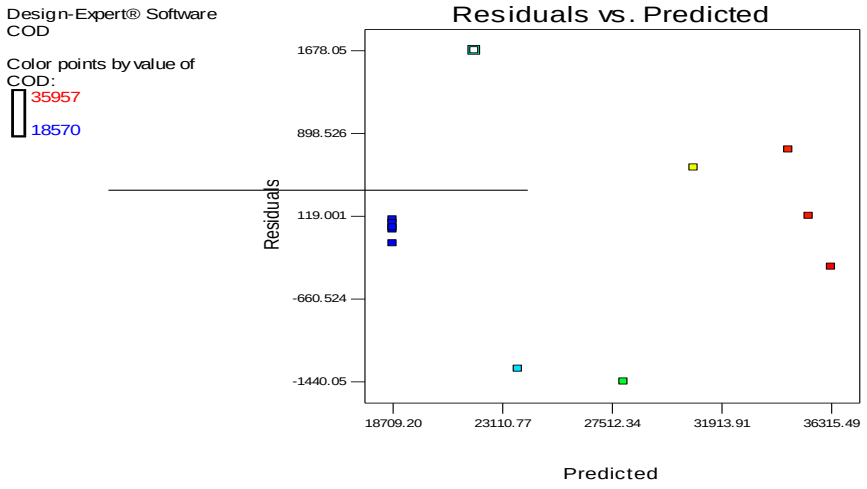
a

Figure 4.1 (a): Predicted vs actual plot of effluent COD



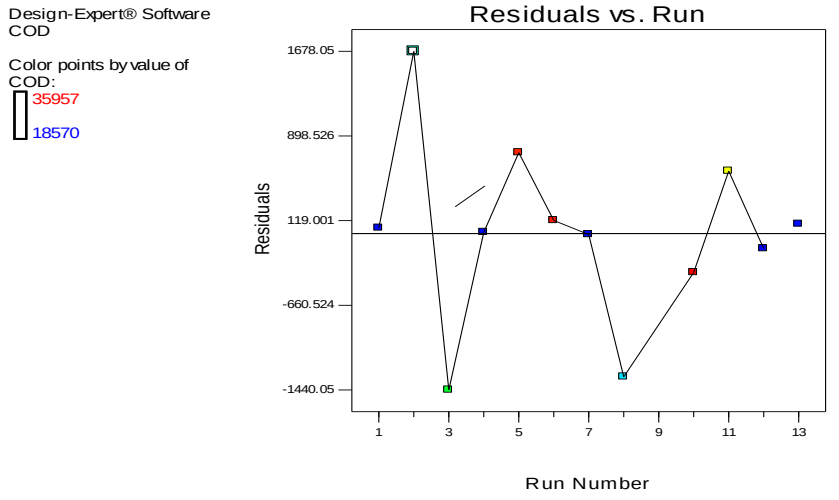
b

Figure 4.1 (b): Residual plot of effluent COD (normal plot vs residuals)



c

Figure 4.1 (c): Residual plot of effluent COD (residuals vs predicted)



d

Figure 4.1 (d): Residual plot of effluent COD (residuals vs run)

The interactive effect of pH and temperature on effluent COD was shown on Figure 4.2.. From the figure it is shown that the effluent COD (18, 570 mg/l) is low at temperature of 35 and pH 7.25. COD removal efficiency at this point is maximum (63.8%). When the pH value decresed to 6.5 the COD removal efficiency decreases (30.8%). Previous research has reported that when the pH falls below 6.5, methanogenic bacteria are inhibited [59]. An excessively alkaline pH could lead to the disintegration of microbial granules and subsequent failure of the process [58]. As the temperature decreases the COD reduction efficency decreases similarly due to unfavarouble condition of microbes.

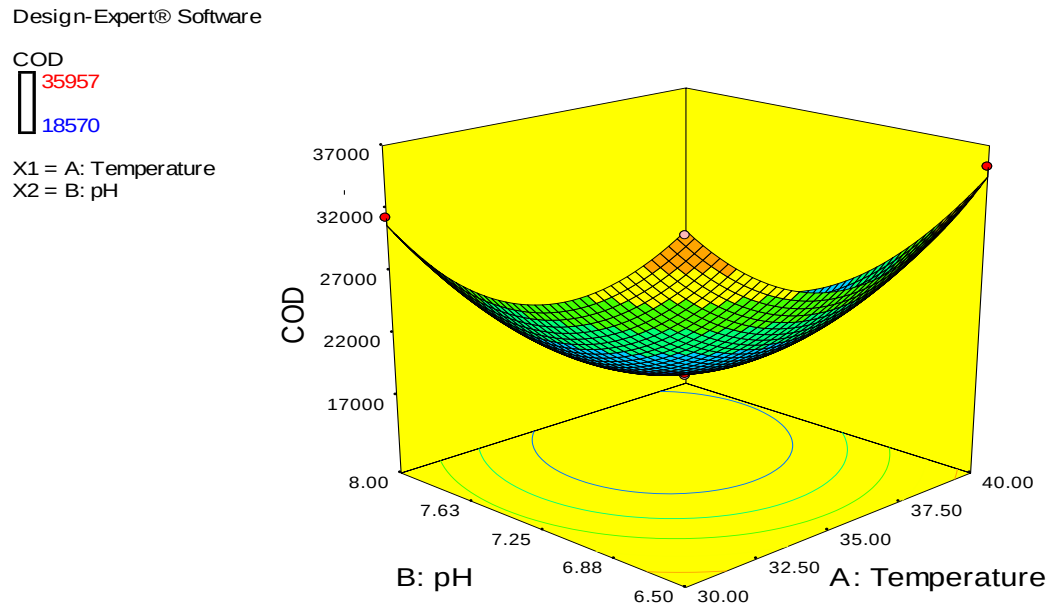


Figure 4.2: 3D plot for interactive effect of pH and Temperature on effluent COD

4.4. The effect of temperature and pH on BOD reduction

The predicted values of BOD were obtained from the quadratic model and by evaluating the relationship between pH and temperature. The statistical model was developed by applying multiple regression analysis using the experimental data of BOD. The final equation in terms of actual factors is given below.

$$BOD = 4.46778E^5 - 8911.44152 \times T - 73961.28757 \times pH - 193.666 \times T \times pH + 143.61848 \times T^2 + 5366.69146 \times pH^2$$

Table 4.6: ANOVA test for BOD

Source	Sum of squares	df	Mean Sqaure	F Value	p-value Prob > F	
Model	162163837.7	5	32432767.5	102.42987	< 0.0001	significant
A-Temperature	13753041.13	1	13753041.1	43.435153	0.0006	
B-pH	23062211.79	1	23062211.8	72.835578	0.0001	
AB	2109756.25	1	2109756.25	6.663078	0.0417	
A^2	83620005.41	1	83620005.4	264.09052	< 0.0001	
B^2	38370255.85	1	38370255.9	121.18178	< 0.0001	
Residual	1899803.279	6	316633.88			
Lack of Fit	1741378.479	2	870689.24	21.98366	0.0070	significant
Pure Error	158424.8	4	39606.2			

The Model F-value of 102.43 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This means both temperature and pH have individual effect on BOD reduction as p value < 0.05. in addition the model terms AB indicates that pH and temperature have combined effect on BOD reduction (p value <0.05). From the ANOVA of the model we can aslo see that the square of each factor (i.e. initial pH & Temperature) has the individual effect on the response (p value < 0.05).

The "Lack of Fit F-value" of 21.98 implies the Lack of Fit is significant. There is only a 0.70% chance that a "Lack of Fit F-value" this large could occur due to noise.

Std. Dev.	562.7023	R-Squared	0.98842
Mean	11179.5	Adj R-Squared	0.978771
C.V. %	5.033341	Pred R-Squared	0.880457
PRESS	19612706	Adeq Precision	23.58959

Table 4.7: Post ANOVA for BOD

The "Pred R-Squared" of 0.8805 is in reasonable agreement with the "Adj R-Squared" of 0.9788. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. the ratio

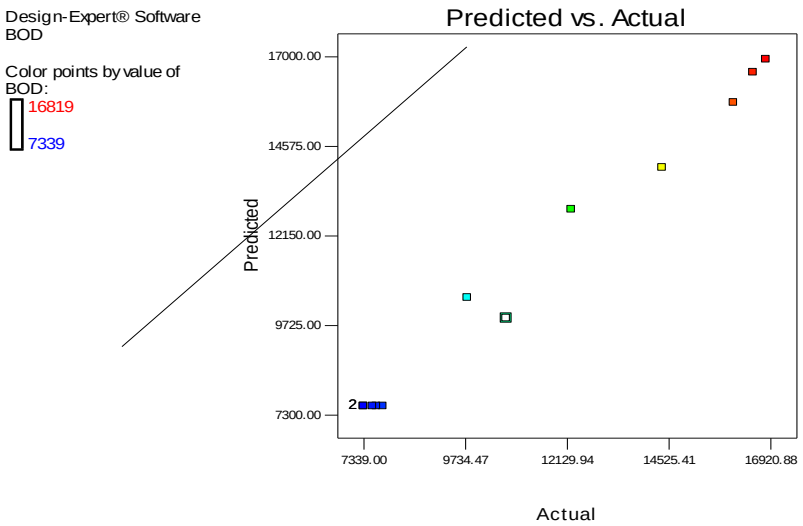
of this work 23.590 indicates an adequate signal. This model can be used to navigate the design space.

The R-squared of 0.98842 showed that the model could explain 98.84 % of the variability in the response

Table 4.8: ANOVA for BOD, standrd error and coefficient of estimate

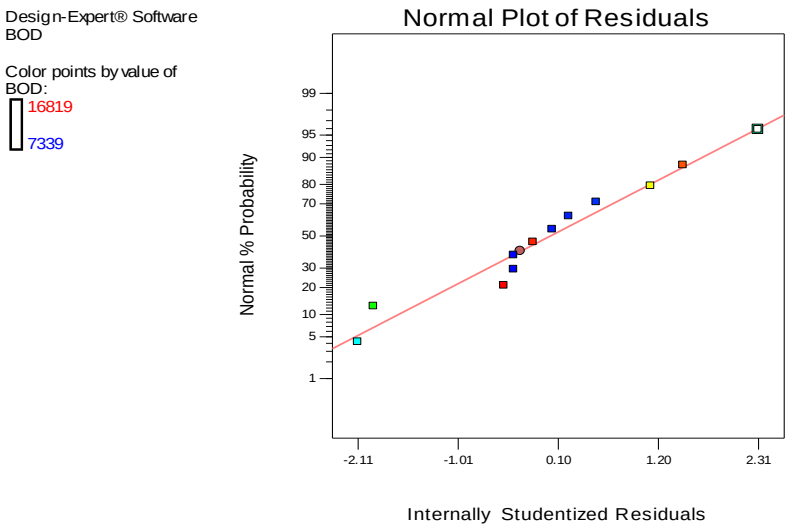
Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	7534.8	1	251.648119	6919.0393	8150.5607
A-Temperature	-1311.156033	1	198.945307	-1797.958	-824.3544
B-pH	-2191.946043	1	256.837286	-2820.404	-1563.488
AB	-726.25	1	281.351151	-1414.691	-37.80856
A^2	3590.462018	1	220.939788	3049.8419	4131.0822
B^2	3018.763947	1	274.22718	2347.7542	3689.7737

Fig 4.3 (a-d), below shows the residual plotes of effluent BOD. Normal probability plot shows that the residuals follow the normal distribition. The residuls vs predicted BOD value follows random scatter which means the model exhibits constant varience. Residuals vs run shows randomscatter.



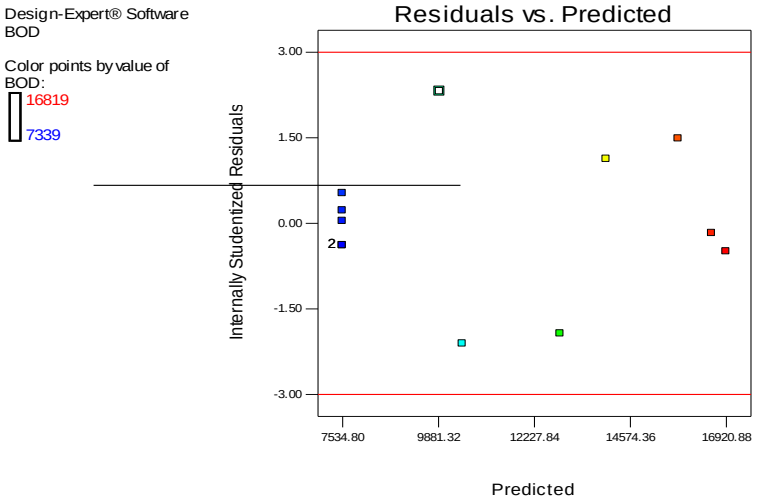
a

Figure 4.3 (a): Predicted vs actual plot of effluent BOD



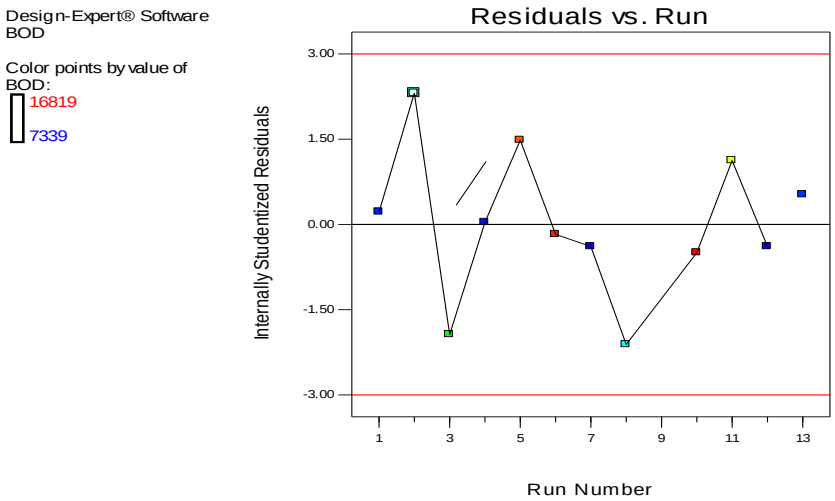
b

Figure 4.3 (b): Residual plot of effluent BOD (normal plot vs residuals)



c

Figure 4.3 (c): Residual plot of effluent BOD (residuals vs predicted)



d

Figure 4.3 (d): Residual plot of effluent BOD (residuals vs run)

Figure 4.4 shows the interactive effect of pH and temperature on effluent BOD. From the figure it is shown that the effluent BOD (7339 mg/l) is lowest at temperature of 35 and pH 7.25. BOD removal efficiency at this point is maximum (76%). When the pH value decresed to 6.5 the BOD removal efficiency decreases (45%). Previous research has reported that when the pH falls below 6.5, methanogenic bacteria are inhibited [59]. An excessively alkaline pH could lead to the disintegration of microbial granules and subsequent failure of the process [58]. As the temperature decreases the BOD reduction efficency decreases similarly due to unfavarouble condition of microbes.

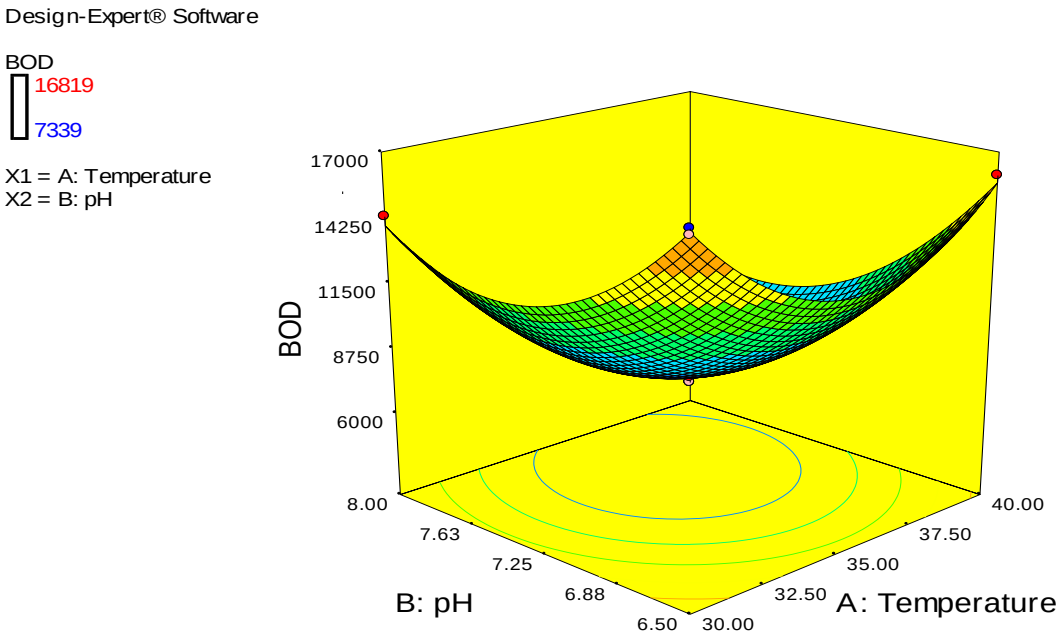


Figure 4.4: 3D plot for the interactive effect of pH and Temperature on effluent BOD

4.5. Effect of pH and Temperature on Methane (CH_4) yield

The predicted values of Methane yield (MY) were obtained from the quadratic model and by evaluating the relationship between pH and temperature. The statistical model was developed by applying multiple regression analysis using the experimental data of MY. The final equation in terms of actual factors is given below.

$$MY = -639.61883 + 13.47637 \times T + 111.49432 \times pH + 0.39336 \times T \times pH - 0.22706 \times T^2 - 8.25843 \times pH^2$$

The Model F-value of 74.94 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Table 4.9: ANOVA for Response Surface Quadratic Model of Methane Yeild

Source	Sum of squares	df	Mean Sqaure	F Value	p-value Prob > F	
Model	431.6134247	5	86.3226849	74.938112	< 0.0001	significant
Intercept						
A-Temperature	37.64445184	1	37.6444518	32.679755	0.0012	
B-pH	82.11254871	1	82.1125487	71.283225	0.0002	
AB	8.70368004	1	8.70368004	7.5558048	0.0333	
A^2	209.0166881	1	209.016688	181.45075	< 0.0001	
B^2	90.86073035	1	90.8607304	78.877663	0.0001	
Residual	6.91151796	6	1.15191966			
Lack of Fit	6.847971852	2	3.42398593	215.52766	< 0.0001	significant
Pure Error	0.063546108	4	0.01588653			

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. This means both temperature and pH have individual effect on methane yeild as p value < 0.05. in addition the model terms AB indicates that pH and temperature have combined effect on methane yeild (p value <0.05). From the ANOVA of the model we can aslo see that the square of each factor (i.e. initial pH & Temperature) has the individual effect MY (p value < 0.05).

Table 4.10: ANOVA for Methane Yeild

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	27.96772	1	0.47998326	26.793243	29.142197
A-Temperature	2.169229467	1	0.37946009	1.2407241	3.0977348
B-pH	4.136034451	1	0.48988087	2.9373392	5.3347297
AB	1.4751	1	0.5366376	0.1619951	2.7882049

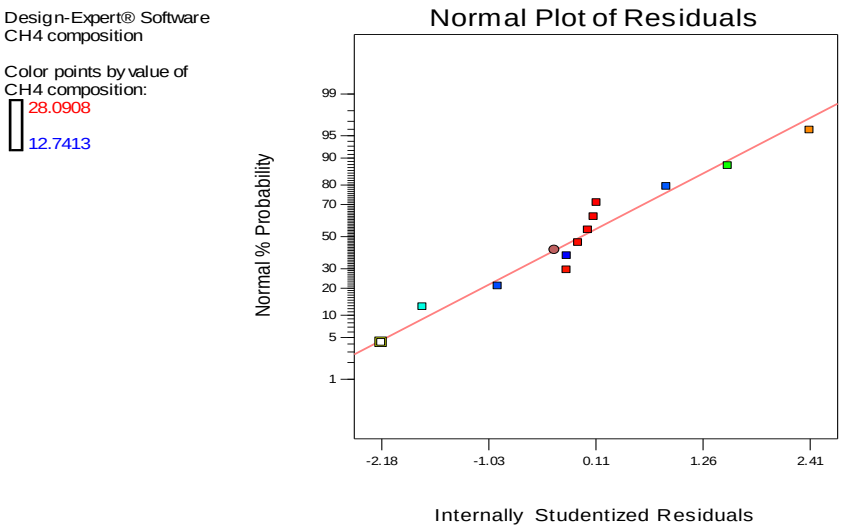
A^2	-5.676566398	1	0.42141145	-6.707723	-4.64541
B^2	-4.645365805	1	0.52304963	-5.925222	-3.36551

The "Pred R-Squared" of 0.8562 is in reasonable agreement with the "Adj R-Squared" of 0.9711. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of this work 19.965 indicates an adequate signal. This model can be used to navigate the design space.

Std. Dev.	1.073275	R-Squared	0.984239
Mean	22.3481	Adj R-Squared	0.971105
C.V. %	4.802535	Pred R-Squared	0.856203
PRESS	63.05839	Adeq Precision	19.96534

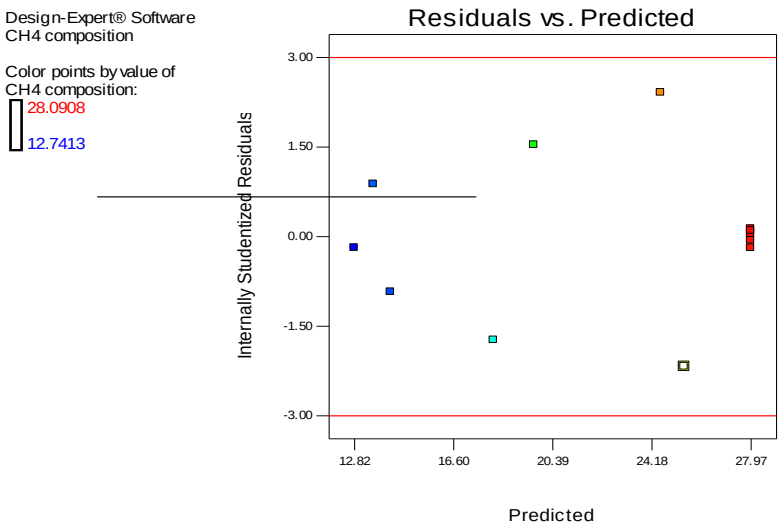
Table 4.11: Post ANOVA for Methane Yeild

Figure 4.5 (a-d) below shows the residual plotes of MY. Normal probability plot shows that the residuals follow the normal distribition. The residuls vs predicted MY value follows random scatter which means the model exhibits constant varience. Residuals vs run shows random scatter.



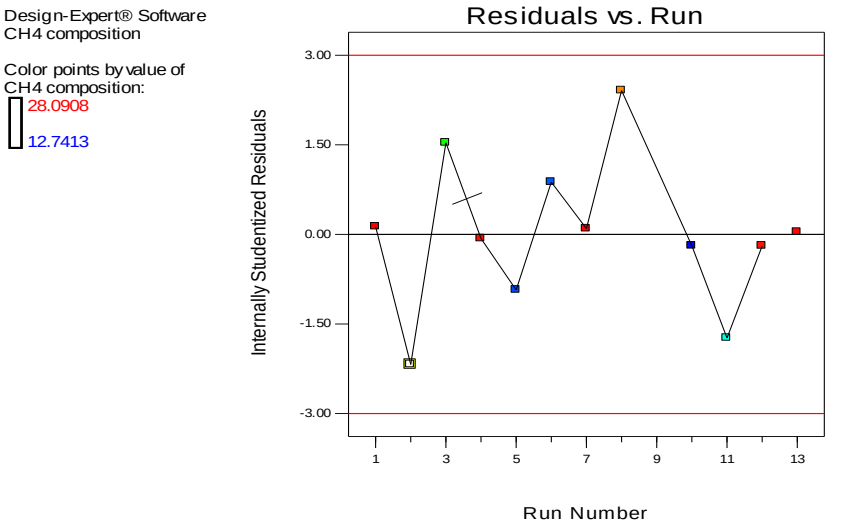
a

Figure 4.5 (a): Normal plot of Methane yield



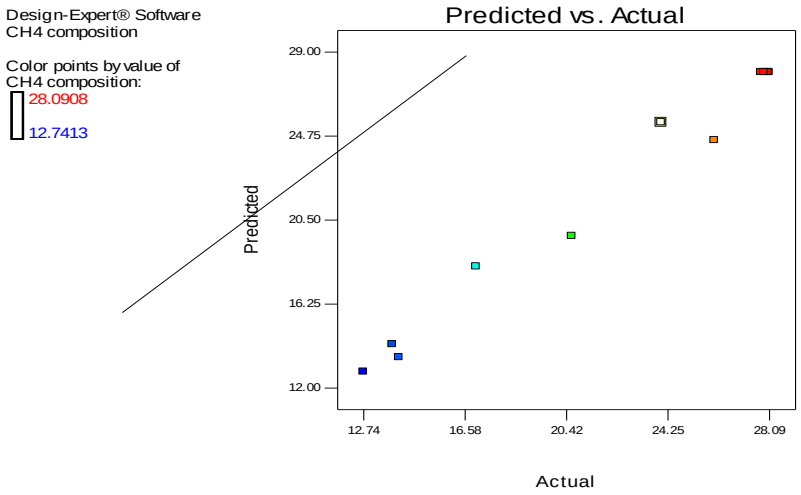
b

Figure 4.5 (b): Residuals vs predicted plot Methane yield



c

Figure 4.5 (c): Residuals vs run of Methane yield



d

Figure 4.5 (d): Predicted vs actual plot of Methane yield

Fig 4.6 shows the interactive effect of pH and temperature on MY. From the figure it is shown that the MY is lowest (12.7413 ml CH4/g COD) at temperature of 30^{o}C and pH 6.5 (Run 10). MY is maximum at a temperature of 35 ^{o}C and initial pH 7.25 (see Fig 4.6). Espinoza-Escalantea et.al 2009, [62] studied biogas production on initial pH of 4.5, 5.5 and 6.5 and stated that Mesophilic temperature (35^{0}C) and initial pH 6.5 has produced the most biogas yield. Budiyono *et al.2013* [56, 67] studied on initial pH at neutral range (6, 7, and 8). After carrying out during 30 days, substrate at initial pH of 7 generated the more biogas than two others, which are pH of 6 and 8. Previous research has reported that when the pH falls below 6.5, methanogenic bacteria are inhibited and biogas production decreased [59]. Buitron and Carjaval, (2010) noted that vinasse could be optimally converted into biogas at temperature digester of 35^{o}C [65]. An excessively alkaline pH could lead to the disintegration of microbial granules and subsequent failure of the process [58]. As the temperature decreases the BOD reduction efficency decreases similarly due to unfavarouble condition of microbes.

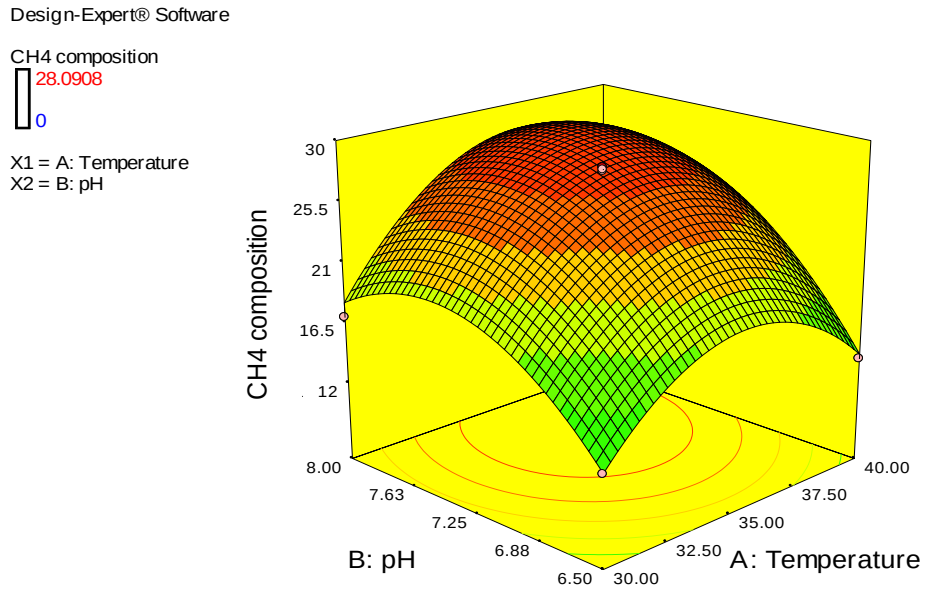


Figure 4.6: 3D plot of the interactive effect of pH and Temperature MY

4.6. Profile of substrate pH after Digestion

During vinasse fermentation in the digester, pH is function of time [23]. At the beginning of vinasse fermentation (in the first four days), pH substrate decreases drastically from 6-8 to 3.7-4.5. Then, pH condition is decreasing until the end fermentation. The pH of digested vinasse at initial pH 7.25 and 35 ^{o}C was around 6.07 – 6.12) due the buffering capacity of vinasse for its high alkalinity value.

Table 4.12: PH profile of substrate after digestion

Run #	Temperature (^{o}C)	pH before Digestion	pH after Digestion
1	35	7.25	6.1
2	40	8	5.9
3	42	7.25	6.07
4	35	7.25	6.12
5	40	6.5	5.07
6	28	7.25	5.6
7	35	7.25	6.12
8	35	8.3	6.20
10	30	6.5	4.85
11	30	8	6.15
12	35	7.25	6.11
13	35	7.25	6.10

Initial pH of 7.25 (fig. 4.7) gives the satisfy result than the others (pH of 6 and 8). Budiyono *et al.* [55] stated that pH of 7 is good condition for anaerobic bacteria to adapt in digester.

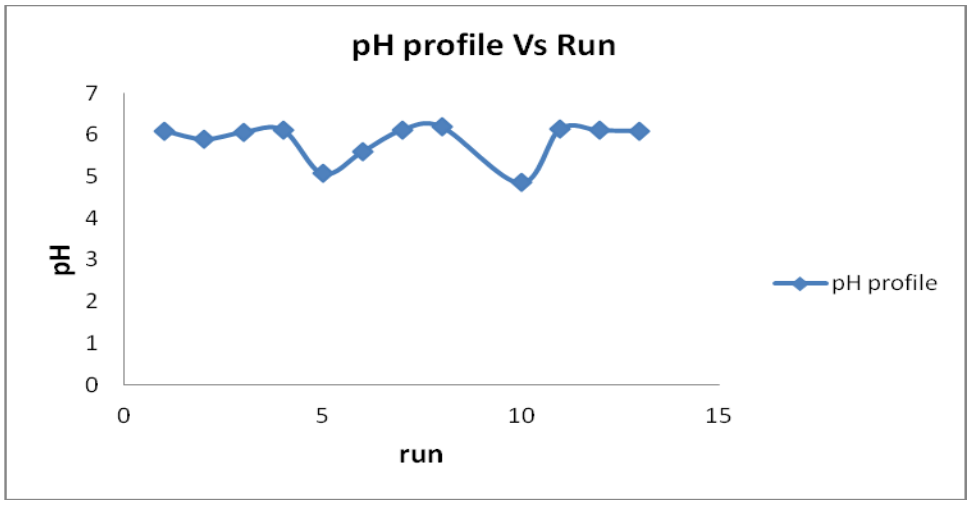


Figure 4.7: Effluent pH profile of experimental runs after digestion

Drop in pH is caused by accumulation of VFAs (Volatile Fatty Acid) in digester [30]. Vinasse is a by-product of ethanol industry that contains variety of organic materials such as acetic acid, lactic acid and glycerol [31]. These are simple organic compound that are easy to be degraded by bacterial activity. This is caused VFAs generated in large amount, so pH drop drastically. Besides that, vinasse also contains high carbohydrate [64]. Substrate contained high carbohydrate generates VFAs easily in anaerobic biotechnology, so the large amount of VFAs will be produced in fermentation of vinasse [56].

4.7. Efficiency of anaerobic digestion

Removal efficiency of main environmental parameters such as COD, BOD and VS after 20 days of anaerobic digestion is shown in the following table. Removal efficiency of each parameter was calculated based on the formulas;

$$\%\ COD\ red = \frac{mass\ of\ COD_{in} - mass\ of\ COD_{out}}{mass\ of\ COD_{in}} \times 100 \quad \text{..... 4.1}$$

$$\%\ BOD\ red = \frac{mass\ of\ BOD_{in} - mass\ of\ BOD_{out}}{mass\ of\ BOD_{in}} \times 100 \quad \text{..... 4.2}$$

$$\%\ VS\ red = \frac{mass\ of\ VS_{in} - mass\ of\ VS_{out}}{mass\ of\ VS_{in}} \times 100 \quad \text{.....4.3}$$

Table 4.13: Removal efficiency of COD, BOD and VS

Run #	Temperature (^{o}C)	pH	COD_{out}	BOD_{out}	VS_{out}	$\%\ COD_{red}$	$\%\ BOD_{red}$	$\%\ VS_{red}$
R 1	35	7.25	18760	7645	18631	63.9	75	52.68
R 2	40	8	23677	10703	21682	54.5	65	44.94
R 3	42	7.25	26549	12232	33476	48.9	60	40.38
R 4	35	7.25	18720	7553	18622	64.0	75.3	52.7
R 5	40	6.5	35345	16054	28974	32.0	47.5	26.41
R 6	28	7.25	35544	16513	29098	31.6	46	26.1
R 7	35	7.25	18700	7339	18595	64.0	76	52.77
R 8	35	8.3	22424	9786	20898	56.9	68	46.93
R 10	30	6.5	35957	16819	29356	30.8	45	25.44
R 11	30	8	31372	14373	26491	39.7	53	32.72

R 12	35	7.25	18570	7339	18600	64.3	76	52.76
R 13	35	7.25	18796	7798	18750	63.8	74.5	52.38

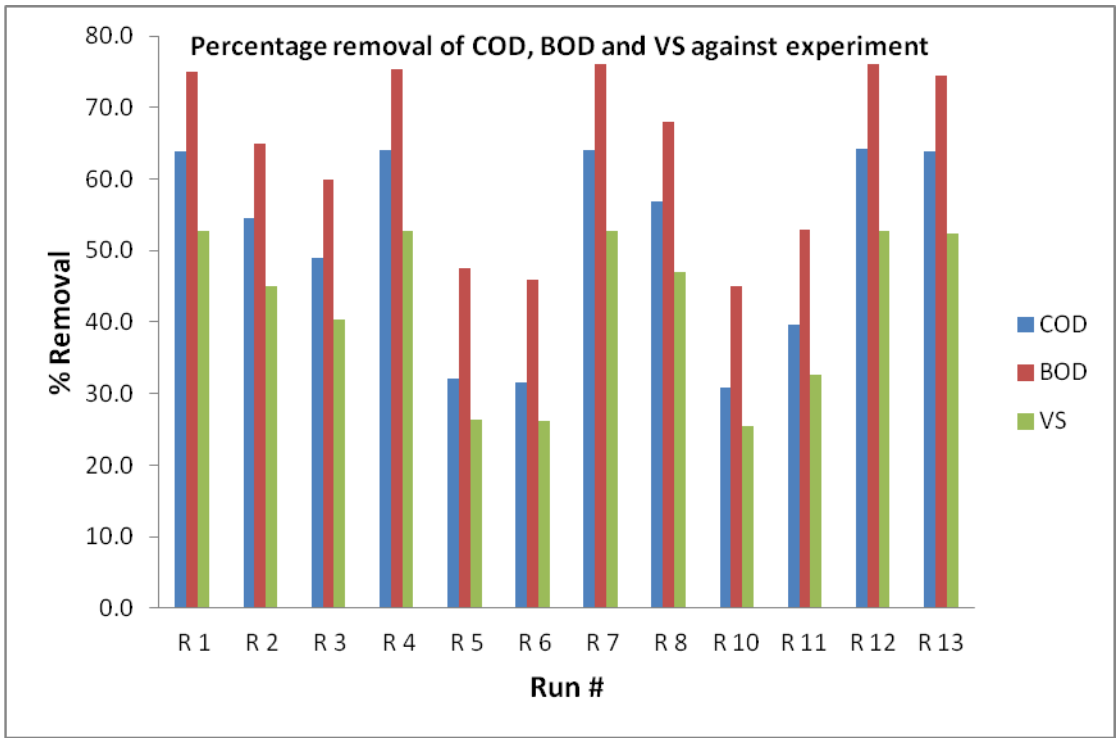


Figure 4.8: Removal efficiency of anaerobic digestion on COD, BOD and VS

As shown in figure 4.8 maximum removal effieciency is shown at temperature of 35 ^{o}C and pH 7.25. the maximum removal effieciencies for COD, BOD and VS at this optimum temperature are 64.3, 75.3 and 52.77 respectively.

4.8. Comparison of digested vinasse with Ethiopian standard

Table 4.14 shows experimental values of vinasse after 20 days of digestion and compares with "Emission Limit Values for Discharges to Water Malting, Brewing, Distilling, Production of Wines and Other Alcoholic liquors" set by Ethiopian EPA. COD, BOD, and total nitrogen values were much higher than the discharge limit. It showed that anaerobic treatment alone cannot be used for purification of vinasse unless followed by aerobic or physico-chemical process based on Espana – Gamboa et al, 2011 [72].

Table 4.14: Comparison of digested vinasse with discharge limit of distilleries based on Ethiopian EPA

Parameter	**Value of Digested Vinasse**	**Discharge Limit Value (mg/l)**
Temperature °C	35	40 °C
pH	6.1	6 – 9
BOD_5 at 20°C, mg/l	7339	60 mg/l
COD, mg/l	18570	250 mg/l
Suspended Solids, mg/l	NA	50
Total Ammonia (as N), mg/l	NA	20
Total Nitrogen (as N) mg/l	62	40 mg/l
Total Phosphorus (as P) mg/l	NA	5 mg/l
Oils, Fats, and Grease mg/l	NA	15
Mineral Oil (Interceptor) mg/l	NA	20

NA - Not Analyzed

4.9. Optimization of pH and Tempereture

Using design expert the optimum values of pH and Temperature for low effluent COD & BOD, high cumulative biogas volume and methane yield as criteria were found.

4.9.1. Numerical optimization

Table 4.14 below shows the high and low values of the experiment. Using the constraints set based on the table the optimum value of each factor (pH and Temperature) and responses (COD, BOD, methane volume and biogas volume) were calculated using design expert 7.0.0 and solution are shown in table 4.15, below.

Table 4.15: Constraints for optimization tool

Name	Goal	Lower limit	Upper limit
temperature ^{o}C	is in range	28	42
pH	is in range	6.5	8
COD (mg/l)	minimize	18570	35957
BOD (mg/l)	minimize	7339	16819
Cumulative Biogas Volume (ml/g COD)	maximize	21.06	34.7
Methane volume (ml/g COD)	maximize	12.7413	28.0908

Table 4.16: Solutions for numerical optimization

Number	Temperature ^{o}C	pH	COD (mg/l)	BOD (mg/l)	Cumulative Biogas Volume (ml/g COD)	Methane volume (ml/g COD)	Desirability	
1	**36.82**	**7.57**	**17448.94**	**7035.595**	**35.41802**	**29.1508**	**1**	**Selected**
2	35.19	7.52	17705.33	7082.912	35.24178	28.95002	1	
3	36.65	7.51	17460.27	7011.855	35.43183	29.11432	1	
4	35.1	7.47	17804.49	7119.761	35.18341	28.84041	1	
5	37.55	7.49	17900.16	7287.754	35.06351	28.69312	1	
6	36.91	7.63	17485.27	7082.989	35.36881	29.15694	1	
7	36.4	7.4	17706.4	7090.86	35.28777	28.85406	1	

Thus Temperature of 36.82 ^{o}C and initial pH of 7.57 is found to be optimum point for minimum effluent COD (17448.94 mg/l), minimum effluent BOD (7035.595), maximum biogas yield (35.41802 ml/g COD) and maximum methane yield (29.1508 ml/g COD).

Contour plot of the optimum point is seen in the figure 4.9, below.

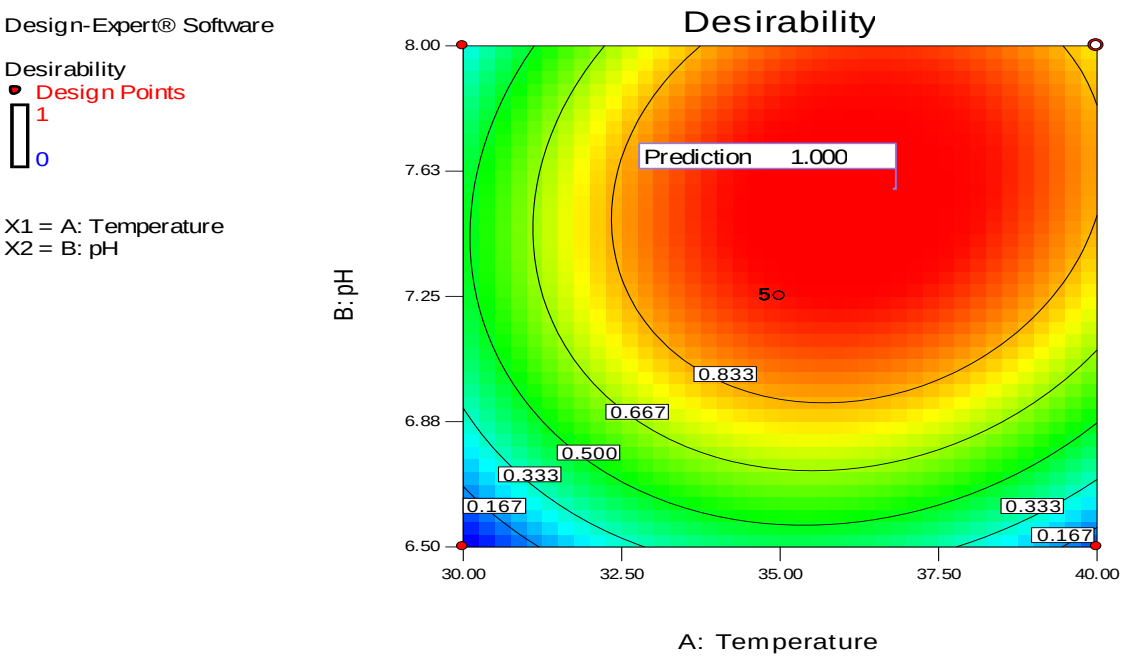


Figure 4.9: Contour plot of optimum point against desirability

4.9.2. Graphical optimization

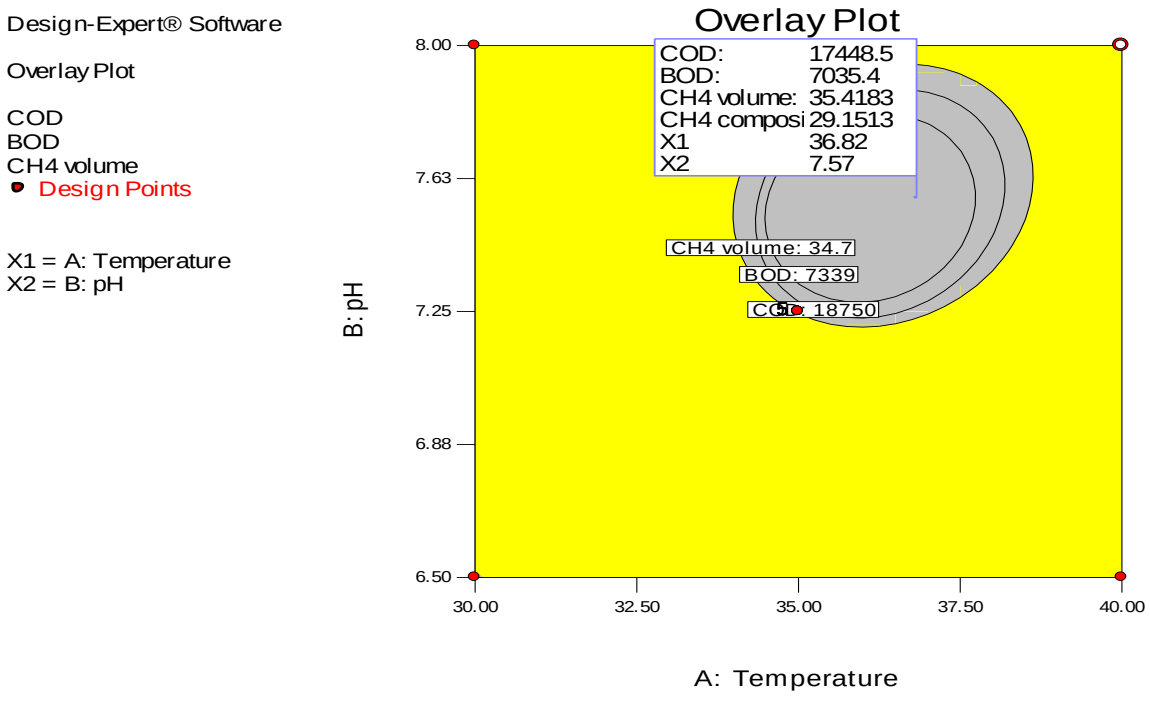


Figure 4.10: Graphical optimization

4.9.3. Point prediction

The optimum design points based on response surface point prediction method is shown in the following table.

Table 4.17: Optimum design points

Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
COD (mg/l)	17448.94	502.6677	16218.96	18678.92	1238.599	14418.2	20479.68
BOD (mg/l)	7035.595	249.8668	6424.193	7646.997	615.6844	5529.07	8542.12
Cumulative Biogas Volume (ml/g COD)	35.41802	0.449081	34.31916	36.51688	1.106559	32.71037	38.12567
Methane volume (ml/g COD)	29.1508	0.476586	27.98464	30.31696	1.174331	26.27731	32.02428

4.10. Effect of pH and Temperature on kinetic model of biogas production

Biogas production for all variables was modeled based on modified Gompertz equation. Kinetic constants y_m, U and λ were determined by using non linear regression. Biogas yield obtained from experimental runs which were measured every two days for 20 days of Hydraulic Retention time (SRT) (Appendix B1 – B7). This result was used as input data to calculate kinetic constants using nonlinear regression and compared with modified Gompertz model (figure 4.11). Runs at 35 ^{o}C and pH 7.25 showed higher result due to the stability of bacterial activity in support of results obtained by other researchers [62]. Kinetic constants obtained are presented in table 4.17, below.

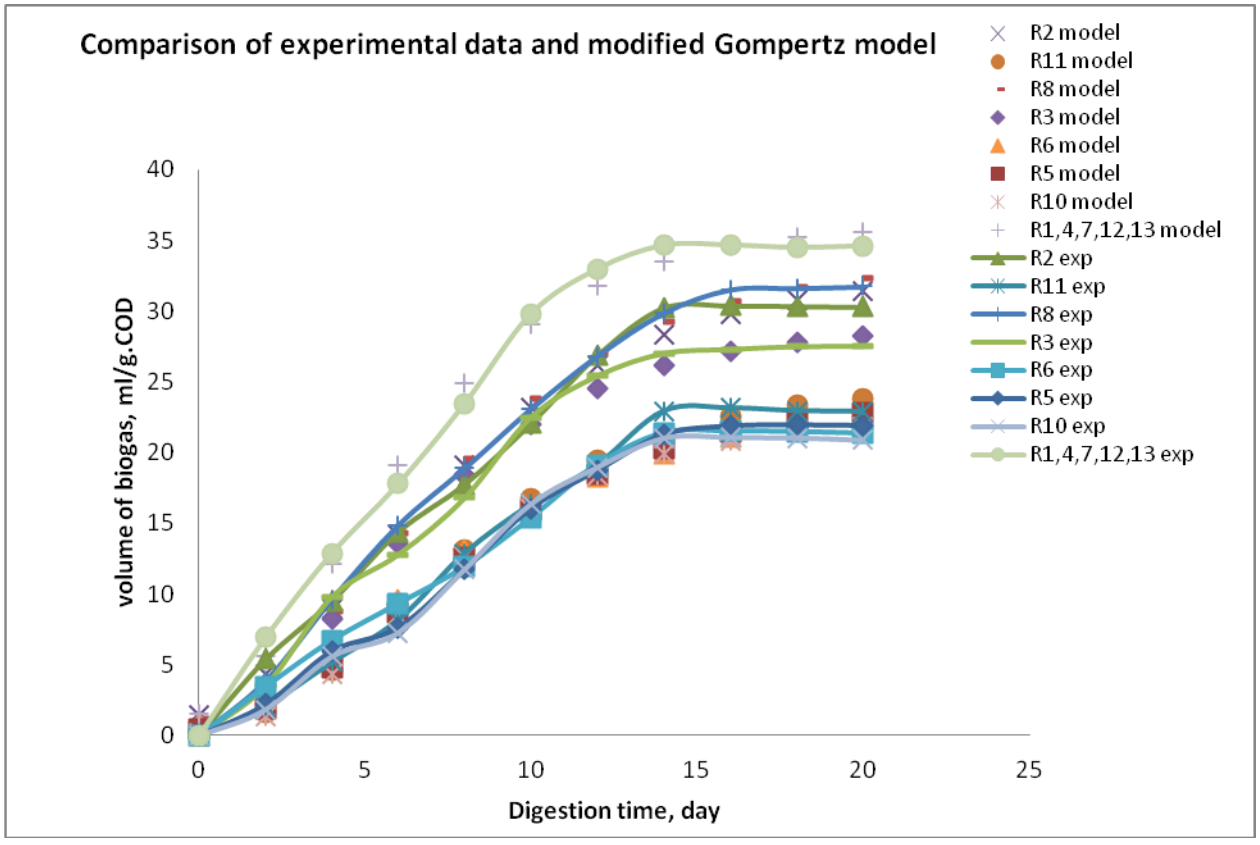


Figure 4.11: Comparison of experimental data and modified Gompertz model

Table 4.18, below shows that pH and Temperature affected the values of kinetic constant. Run 10 (T= 30 ^{o}C and pH=6.5) has lowest value of y_m (22.333 ml/g COD). That means Run 10 in prediction generated low biogas. Whereas, variable with R1, 4, 7, 12, 13 (T=35^{o}C and pH=7.25)

has highest value of y_m (36.110 ml/g COD). Substrate with a temperature of 35 ^oC and pH of 7.25 caused favarouble condition for bacterial growth in the digester, as a result the generated biogas became high relative to the other runs.

Kinetic constant of y_m describes maximum biogas that can be produced. Vinasse fermentation produces maximum biogas in little amount, which is 39.406 – 140.164 mL/g VS. The result of this study is 45.79 ml/g VS (36.11 ml/g COD). This figure is lower than other substrates such as cattle manure (418.26 ml/g VS) and municipal solid waste (522 ml/g VS) due the presence of phenolic compounds in vinasse.

Run 10 had highest value of λ (2.089) meaning bacteria in this variable took them long to adapt and produce biogas. Zwietering et al, 1990 [60], reported that value of λ (0.597) indicated the time that was required for bacteria to adapt. Variable at 35 ^oC and pH 7.25 had little value of λ implying needed little time to produce biogas. Budiyono et al, 2010 [61], stated that variable that had little value of kinetic constant of λ, needed little time to produce biogas. Espinoza-Escalantea et.al 2009, [62] stated that Mesophilic temperature (35^0C) and initial neutral pH has produced the most biogas yield.

	Experiment	Modified Gompertz equation			
Variable	Biogas volume (ml/g COD)	y_m (ml/g COD)	U (ml/g COD. day)	λ (day)	R^2
R1,4,7,12,13	34.680	36.110	3.560	0.597	0.9917
R2	30.390	32.630	2.650	0.650	0.9900
R3	27.510	28.810	2.710	0.986	0.9917
R5	21.970	23.701	2.018	1.711	0.9920
R6	21.510	23.285	1.866	0.852	0.9876
R8	31.730	33.447	2.822	0.915	0.9963
R10	21.060	22.333	2.178	2.089	0.9893
R11	23.140	24.662	2.210	2.013	0.9933

Table 4.18: Kinetic constant of biogas production based on modified Gompertz model.

Prediction of biogas production from anaerobic digestion of vinasse was made by using modified Gompertz equation. Some authors make prediction of biogas production through modified Gompertz equation because of its good correlation coefficient (R^2), which based on Budiyono et al. [63] is 0.993 – 0.999, Syaichurrozi et al. [64] is 0.958 - 0.967, Budiyono et al. [54] is 0.986-0.998. The correlation coefficient of this study is 0.9876 – 0.9963 which is in the range of previous studies.

4.11. Design of UASB reactor

Anaerobic digestion using vinasse as substrate for vinasse treatment and biogas production has been performed by UASB successfully in Brazil [69]. Upflow Anaerobic sludge blanket reactor (UASB) has a bottom sludge bed, dense and granular anaerobic biomass. Mixing is provided by the upflow velocity and biogas generation [12]. UASB is designed for higher COD loading (5 – 20 kg/m^3.day) [12]. As vinasse is known for its high organic load UASB is preferred reactor for its anaerobic treatment. Thus UASB reactor is selected for the case study taken in this thesis work. UASB reactor has 90 -95 % COD removal capacity there by producing large amount of methane gas as alternative renewable energy.

Design data: Design parameters are given in the following table

Table 4.19: Design data for UASB reactor

Item	Symbol	Unit	Value
Flow rate	Q	m^3/day	360
Influent COD	S_o	mg/l	51987
Influent BOD		mg/l	30581
Synthesis yield	Y	g VSS/g COD	0.08*
Decay coefficient	k_d	g VSS/g COD. day	0.03*
Temperature	T	^oC	35
Methane production at 35 ^oC	MY	m^3/kg COD	0.4
Methane density at 35 ^oC		Kg/m^3	0.6346
Energy content of Methane		KJ/g	50.1
Methane Composition		%	81**

Note: ** maximum methane composition obtained at (35 ^oC and pH 7.25) of this thesis work.

All kinetic coefficients (Y, k_d,) are based on Metcalf and eddy [6]

Basic assumptions (based on Metcalf and eddy) [6]

1. COD removal efficiency – 90 %
2. Reactor Volume effectiveness factor (E) – 0.9
3. Height of gas collection – 2.5 m
4. Height of reactor – 8 m
5. Up flow velocity (soluble COD) – 1 m/h
6. Organic loading rate – 18000 g COD/m^3. day
7. SRT (Solids retention time) – 20 days.

Determination of reactor volume

To determine the reactor volume and dimensions the organic loading, superficial velocity and effective treatment volume must be considered. Effective treatment volume is the volume occupied by the sludge blanket and active biomass.

$$V_n = \frac{Q \times S_o}{L_{org}}$$

Where, Vn = effective liquid volume of reactor, m^3

Q = influent flowrate, m^3/h

So = influent COD, kg COD/m^3

Lorg = organic loading rate, kg $COD/m^3.d$

Substituting the values from table XX, effective volume is

$$V_n = \frac{360\,\frac{m3}{day} \times 51.987\ kg/m3}{18\ kg\ COD/m3.d} = 1039\ m^3$$

Determination of total reactor volume

To determine total reactor liquid volume below the gas collector's effectiveness factor E is used, which is the fraction occupied by the sludge blanket.

$$V_l = \frac{Vn}{E}$$

Where V_l = total reactor volume

E = effectiveness factor

Taking effectiveness factor of 0.9, the total reactor volume is:

$$V_l = \frac{Vn}{E} = \frac{1039}{0.9} = 1094\ m^3$$

Determination of area of the reactor

The area of the reactor can be calculated by the relationship.

$$A = \frac{V_l}{H} \qquad \text{..... 4.4}$$

Substituting the values for each parameter in equation 4.4, area of the reactor is:

$$A = \frac{V_l}{H} = \frac{1094}{8} = 137\ m^2$$

Determination of total height of the reactor

The gas collection volume is in additional to reactor volume and adds an additional height of 2 – 3 meter based on Metcalf and Eddy [6]. Taking typical value of 2.5 m,

$$H_t = H + H_g \quad \text{..... 4.5}$$

$$H_t = 8 + 2.5 = 10.5$$

Determination of Diameter of the reactor

$$D = \sqrt{\frac{4 \times A}{3.14}} \quad \text{..... 4.6}$$

Substituting the value of calculated area in equation 4.6:

$$D = \sqrt{\frac{4 \times 137}{3.14}} = 13\ m$$

Determination of the reactor hydraulic detention time

$$HRT = \frac{V_n}{Q} \quad \text{..... 4.7}$$

Substituting the values of Q (flow rate) and effective volume Vn in equation 4.7 above,

$$HRT = \frac{1094\ m^3\ \times 24\ h/day}{360 m^3/day} = 72\ h$$

Determination of quantity of cell tissue produced per day

Assume non biodegradable volatile suspended solids are equal to effluent VSS, (nbVSS = Xe)

$$P_x = \frac{Y\left[(So-S)\times Q\times\frac{1kg}{1000g}\right]}{1+k_d\theta_c} + \frac{Y\times k_d\times f_d\left[(So-S)\times Q\times\frac{1kg}{1000g}\right]}{1+k_d\theta_c} + Q\times nbVSS - QX_e$$

$$P_x = \frac{0.08\ kg\ VSS/kg.COD\ \left[(51987\ g/m^3 - 0.1\times 51987\ g/m^3)\times 360\ m^3/day\times\frac{1kg}{1000g}\right]}{1+\ 0.03*30} + \frac{0.08\ kg\ VSS/kg.COD\times 0.038\times 0.15\ \left[(51987\ g/m^3 - 0.1\times 51987\ g/m^3)\times 360\ m^3/day\times\frac{1kg}{1000g}\right]}{1+\ 0.03*30}$$

$$= 805\ kg\ VSS/day$$

Calculation of biogas Production

$$V_{CH4} = 0.35\left[(So-S)\times\frac{(Q)1kg}{1000g}\times -1.42P_x\right]$$

Substituting the values each parameter in the equation, volume of methane produced daily is

$$V_{CH4} = 0.4\frac{m^3}{kg}\left[(51987 - 0.1*51987)\times\frac{(360)m^3 1kg}{1000g.day}\times -1.42*805\ kg/day\right]$$
$$= 6280m^3/day$$

Total biogas production

$$Total\ Volume_{biogas} = \frac{V_{CH4}}{0.81} = \frac{6280}{0.81} = 7753\ m^3/day$$

Energy content of Gas

At 35 ^oC the volume occupied by 1 mole of gas is

$$V = \frac{n\times R\times T}{P}$$

$$V = \frac{1\ mole\times 0.082057\ at.\frac{l}{molK}\times(273.15+35)}{1\ atm} = 25.28\ l$$

Total mass of methane produced per day

$$mole\frac{CH4}{day} = \frac{\frac{6280m^3}{day}}{\frac{25.28\frac{l}{mole}}{1000}\frac{m^3}{mole}} = 248400\ mole/day$$

Mass of methane

$$mass\ methane = mole\ of\ methane/day \times molar\ mass\ of\ methane$$

$$mass\ methane = 248400\frac{mole}{day} \times 16\frac{g}{mole} = 3974400\ g/day$$

Energy content

$$Enery\ content\ of\ methane = 3974400\frac{g}{day} \times \frac{50.1\ KJ}{g} = 199{,}117{,}440\ KJ/day$$

Thus treatment of vinasse by anaerobic digestion produces considerable energy of 1.99 x 10^8 KJ/day is generated by production of methane, which is equivalent to 10850 KWH per day, 6280 liter of fuel oil per day and 14306.4 kg/day of bagasse. This amount of energy is avoids purchasing of electricity from national grid to supplement the energy shortage of the distillery plant. The surplus energy can be directly fired with bagasse thereby saving bagasse which will be available for sell to paper and particle board factories.

The Design Summary is shown in the following table 4.20.

Table 4.20: Design Summary

Design Parameter	**Value**	**Unit**
Mass flow rate of digester	360	M^3 /day
Volume of digester	1094	M^3
Influent COD	51987	mg/l
Effluent COD	5198.7	mg/l
Organic loading rate	18	Kg COD/m^3.day
Surface area	137	m^2
Diameter	13	M
Liquid Height	8	M
Total Height	10.5	M
Upflow velocity	1	m/h
Solids retention time (SRT)	30	Day
Hydraulic retention time (HRT)	72	Hour
Solids produced	805	Kg VSS/day
Volume of methane produced	6280	M^3/kg COD
Volume of biogas produced	7753	M^3/kg COD
Energy gained	1.99×10^8	KJ/day

Chapter 5: Conclusion and Recommendation

Conclusion: Vinasse is a dark brown wastewater produced in large amounts in ethanol production from sugar cane processed. The fermentation of sugar cane and the subsequent distillation of ethanol generate between 10 and 15 liters of vinasse per liter of ethanol produced. Disposal of vinasse directly to the environment without treatment causes huge environmental problems. Application of anaerobic digestion to vinasse treatment is a preferable primary treatment option, due to the conversion of organic matter into biogas for subsequent recovery of energy. The combined effect of temperature and pH on biogas production and reduction of organic load such as COD, BOD and VS was studied in this work. Accordingly temperature of 35 ^{o}C and initial pH of 7.25 has produced 34.68 ml of CH4/g COD. In addition at this point 64, 76 and 52.77 % of reduction observed in COD, BOD and VS. The minimum amount of biogas production (21.05 ml of CH4/g COD) observed at a temperature of 30 ^{o}C and pH of 6.5. The removal efficiency of 30.8, 45, and 25.4 % is obtained for COD, BOD and VS at temperature of 30 ^{o}C and pH of 6.5. From the results of this experiment we can conclude that temperature and pH have direct impact on the production of biogas from anaerobic digestion of vinasse and reduction of organic matter. It is also noted that vinasse is a suitable feedstock for anaerobic digestion and can be a good source of alternative green energy. From the design of UASB reactor, the expected amount of energy generated from anaerobic digestion of vinasse of Metehara sugar factory distillery plant is 1.99 x 10^{8} KJ/day. This energy is equivalent to 10850 KWH per day 6280 liter of fuel oil per day and 14306.4 kg/day of bagasse. With the ever increasing of energy prices, the methane produced could be used for the reduction of production costs of the distillery.

The sludge left after anaerobic digestion still possesses considerable amount of organic matter and doesn't meet environmental standards set by Ethiopian EPA.

Recommendation: The effluent discharge has high amount of organic suspended mater. This shows that single stage anaerobic digestion alone can't be used for mere treatment of vinasse. Thus the sludge has to be treated in anaerobic followed by aerobic to provide effluent polishing either in the form of attached or suspended growth and physicochemical treatment such as adsorption and flocculation to meet environmental standards set by regulatory bodies.

Recommendation for future work

- Co digestion: Anaerobic digestion appears to become more stable and productive when varieties of substrates are co digested. Sugar industry has wastes which have high organic content such as filter cake and bagasse. Co digestion of vinasse along with filter cake and bagasse may produce better biogas yield and the writer of this thesis hence recommends for the future work.

- Evaluation of vinasse treatment by using second stage anaerobic digestion and evaluation of its economic viability

- Fertilizer: the suitability of sludge discharged daily after anaerobic digestion should be evaluated against the soil requirement and fertilizer standards.

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Appendix A1: Determination of Total Solids

Procedure

a. Preparation of evaporating dish:

1. If volatile solids are to be measured ignite clean evaporating dish at 550°C for 1 h in a muffle furnace.
2. If only total solids are to be measured, heat clean dish to 103 to 105°C for 1 h.
3. Store and cool dish in desiccator until needed.
4. Weigh immediately before use.

b. *Sample analysis:*

1. Choose a sample volume that will yield a residue between 2.5 and 200 mg.
2. Pipet a measured volume of well-mixed sample, during mixing, to a preweighed dish. For homogeneous samples, pipet from the approximate midpoint of the container but not in the vortex. Choose a point both middepth and midway between wall and vortex.
3. Evaporate to dryness on a steam bath or in a drying oven. Stir sample with a magnetic stirrer during transfer. If necessary, add successive sample portions to the same dish after evaporation. When evaporating in a drying oven, lower temperature to approximately 2°C below boiling to prevent splattering.
4. Dry evaporated sample for at least 1 h in an oven at 103 to 105°C
5. Cool dish in desiccators to balance temperature, and weigh.
6. Repeat cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less.

Calculation

$$Total\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{.....3.1}$$

Where A – weight of dried residue + dish, mg, B – weight of dish, mg

$$Volatile\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{.....3.2}$$

Where, A= weight of residue + dish before ignition, mg, B = weight of residue + dish or filter after ignition, mg.

Appendix A2: Determination of Total Dissolved Solids

Procedure

a. Preparation of glass-fiber filter disk:

4. Insert disk with wrinkled side up into filtration apparatus.
5. Apply vacuum and wash disk with three successive 20-mL volumes of reagent-grade water. Continue suction to remove all traces of water.
6. Discard washings.

b. Preparation of evaporating dish:

1. If volatile solids are to be measured, ignite cleaned evaporating dish at 550°C for 1 h in a muffle furnace.
2. If only total dissolved solids are to be measured, heat clean dish to 180 ± 2°C for 1 h in an oven.
3. Store in desiccator until needed.
4. Weigh immediately before use.

c. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If more than 10 min are required to complete filtration, increase filter size or decrease sample volume.

d. Sample analysis:

1. Stir sample with a magnetic stirrer and pipet a measured volume onto a glass-fiber filter with applied vacuum.
2. Wash with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Transfer total filtrate (with washings) to a weighed evaporating dish and evaporate to dryness on a steam bath or in a drying oven. If necessary, add successive portions to the same dish after evaporation.
3. Dry evaporated sample for at least 1 h in an oven at 180 ± 2°C, cool in a desiccator to balance temperature, and weigh.

4. Repeat drying cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of previous weight or 0.5 mg, whichever is less. Analyze at least 10% of all samples in duplicate. Duplicate determinations should agree within 5% of their average weight.

Calculation

$$Total\ Dissolved\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{..... 3.3}$$

Where A – weight of dried residue + dish, mg, B – weight of dish, mg

Appendix A3: Determination of Total Suspended Solids

Procedure

a. Preparation of glass-fiber filter disk:
   1. Insert disk with wrinkled side up in filtration apparatus. Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water. Continue suction to remove all traces of water,
   2. Turn vacuum off and discard washings.
   3. Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish.
   4. Dry in an oven at 103 to 105°C for 1 h. If volatile solids are to be measured, ignite at 550°C for 15 min in a muffle furnace.
   5. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less.
   6. Store in desiccator until needed.
b. Selection of filter and sample sizes: Choose sample volume to yield between 2.5 and 200 mg dried residue. If volume filtered fails to meet minimum yield, increase sample volume up to 1 L. If complete filtration takes more than 10 min, increase filter diameter or decrease sample volume.
c. Sample analysis:

1. Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of reagent-grade water to seat it
2. Stir sample with a magnetic stirrer at a speed to shear larger particles, While stirring, pipet a measured volume onto the seated glass-fiber filter.
3. Wash filter with three successive 10-mL volumes of reagent-grade water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete. Samples with high dissolved solids may require additional washings.
4. Carefully remove filter from filtration apparatus and transfer to an aluminum weighing dish as a support.
5. Dry for at least 1 h at 103 to 105°C in an oven,
6. Cool in a desiccator to balance temperature, and weigh.

Calculation

$$Total\ Suspended\ Solids\ (\frac{mg}{l}) = \frac{(A-B)\times 1000}{Sample\ Volume, mL} \quad \text{..... 3.4}$$

Where, A = weight of filter + dried residue, mg, and B = weight of filter, mg

Appendix A4: Determination of COD

Apparatus used

a. Digestion vessels: Preferably use borosilicate culture tubes, 16 × 100 mm, 20 × 150 mm, or 25 × 150 mm, with tetra-fluoro-ethylene (TFE) lined screw caps.
b. Block heater or similar device to operate at a temperature of 150 ± 2 °C, with holes to accommodate digestion vessels. Do not use an oven because of the possibility of leaking samples generating a corrosive and possibly explosive atmosphere.
c. Microburet
d. Ampule sealer: Use only a mechanical sealer to insure strong and consistent seals.

Reagent used

a. Standard potassium dichromate digestion solution, 0.01667M: Add to about 500 ml distilled water 4.903 g $K_2Cr_2O_7$, primary standard grade, previously dried at 150 0C for 2 hr, 167 ml H_2SO_4, and 33.3 g $HgSO_4$. Dissolve, cool to room temperature, and dilute to 1000 ml.
b. Sulfuric acid reagent: Add Ag_2SO_4, reagent or technical grade, crystals or powder, to H_2SO_4 at the rate of 5.5 g Ag_2SO_4/kg H_2SO_4.
c. Ferroin indicator solution: Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $FeSO_4 \cdot 7H_2O$ in distilled water and dilute this reagent by a factor of 5 (1 + 4).
d. Standard ferrous ammonium sulfate titrant (FAS), approximately 0.10 M: Dissolve 39.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 ml H_2SO_4, cool, and dilute to 1000 ml.
e. Standardize solution daily against standard $K_2Cr_2O_7$ digestion solution as follows: Pipet 5.00 ml digestion solution into a small beaker

$$\text{Molarity of FAS solution} = \frac{\text{Vol. of 0.01667M } K_2Cr_2O_7 \text{ solution titrated, ml}}{\text{Vol. of FAS used in titration, ml}} \times 0.1 \quad \text{..... .. 3.5}$$

f. Sulfamic acid: Required only if the interference of nitrites is to be eliminated.
e. Potassium hydrogen phthalate (KHP) standard, $HOOCC_6H_4COOK$: Lightly crush and then dry KHP to constant weight at a temperature of 110 0C. Dissolve 425 mg in distilled water and dilute to 1000 ml

Experimental procedure

a. Wash culture tubes and caps with 20 % H_2SO_4 before first use to prevent contamination.
b. Place tubes or ampules in block digester preheated to 150 °C and reflux for 2 hrs behind a protective shield.
c. Cool to room temperature and place vessels in test tube rack.
d. Remove culture tube caps and add small tetraflouro ethylene (TFE) covered magnetic stirring bar. If ampules are used, transfer contents to a larger container for titrating
e. Add 0.05 to 0.10 ml (1 to 2 drops) ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10 M FAS.

f. The end point is a sharp color change from blue-green to reddish brown, although the bluegreen may reappear within minutes. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.

Calculation

Chemical oxygen demand (COD) concentration was calculated using the following formula:

$$COD\left(\frac{mg}{l}\right) = \frac{(FASs - FASp) \times N \times f}{Vs} \quad \text{……..……. 3.6}$$

Where, FASs: used ferrous ammonium sulphate concentration for sample, mg/l, FASp: used ferrous ammonium sulphate concentration for pure water, mg/l, f: dilution factor, N: normality of FAS and Vs: sample volume, ml

Determination of BOD_5

Experimental procedures

Preparation of the sample

a. Select the volume for the wastewater sample
b. The sample volume is related to the expected BOD5 value. The BOD5 incubator is designed to operate with the following BOD5 ranges and sample volume allowing BOD5 measurement.
    i. BOD5 range 0 - 400 mg/l use the sample without dilution
    ii. BOD5 range 0 - 2000 mg/l, the expected sample volume is 56 ml with 3 drop of nitrification inhibitor and 3 - 4 drop of potassium hydroxide (KOH) addition.
    iii. BOD5 range 0 - 4000 mg/l the expected sample volume is 21.2 ml with 1 drop of nitrification inhibitor and 3 - 4 drop of potassium hydroxide (KOH) addition.
c. Carry out the necessary pretreatment of the wastewater sample, setting pH between 6.5 - 7.5, if higher or lower adjust by HCl and NaOH and mix well and allow the sample to settle and filtrate of the sample
d. Measure the wastewater sample precisely using appropriate overflow and if necessary add nitrification inhibitor
e. Insert magnetic stirring rod

f. Place 3 - 4 drop of KOH solution into the seal gasket and insert gasket in the neck of the bottle, screw the BOD sensors to the sample bottle and then place the bottle in the bottle rack
g. Finally, incubate the sample for 5 days at a temperature of 20 0C.

Calculation

For each test bottle meeting the 2.0 mg/l minimum dissolved oxygen (DO) depletion and the 1.0 mg/l residual DO, calculate BOD5 as follows:

$$BOD5\left(\frac{mg}{l}\right) = \frac{D1 - D2}{P}$$

Where;

D1 = DO of diluted sample immediately after preparation, mg/l,

D2 = DO of diluted sample after 5 day incubation at 20 °C, mg/l,

Appendix B1: Experimental biogas production at T=35 oC and pH=7.25 average of (R1, 4, 7, 12, 13)

Time	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	1.4862675
2	560	200	360	6.92	9.142857143	9.2	0.63708235	5.5904417
4	920	250	670	12.89	17.01587302	20	2.57756747	12.130491
6	1200	275	925	17.79	23.49206349	30	5.33787293	19.081183
8	1510	290	1220	23.47	30.98412698	38	8.91761402	24.867967
10	1850	300	1550	29.82	39.36507937	49.5	14.7584973	29.033965
12	2020	305	1715	32.99	43.55555556	65	21.4428607	31.786289
14	2110	308	1802	34.66	45.76507937	78	27.0367592	33.515049
16	2110	307	1803	34.68	45.79047619	81	28.0922154	34.569174
18	2100	306	1794	34.51	45.56190476	75	25.8814704	35.200863
20	2100	300	1800	34.62	45.71428571	72	24.9293093	35.575575

Appendix B2: Experimental biogas production at T=40 oC and pH=8.0 (R2)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	1.4171483
2	479.44	200	279.44	5.38	7.096888889	10	0.537519	4.3510547
4	744.01	250	494.01	9.50	12.54628571	21	1.99553927	8.9444888
6	1023.5	275	748.5	14.40	19.00952381	32	4.60730567	14.210186
8	1213.15	290	923.15	17.76	23.44507937	41	7.28050282	19.131514
10	1447.7	300	1147.7	22.08	29.14793651	55	12.1421702	23.158703
12	1702.2	305	1397.2	26.88	35.48444444	79.3	21.3126282	26.182456
14	1879.85	308	1571.85	30.24	39.92	74.9	22.6463472	28.330061
16	1887	307	1580	30.39	40.12698413	74.6	22.6725912	29.801713
18	1884	306	1578	30.35	40.07619048	72.5	22.0064632	30.787176
20	1875	300	1575	30.30	40	69.1	20.9345606	31.437356

Appendix B3: Experimental biogas production at T=42 ^{o}C and pH=7.25 (R3)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	0.86907131
2	360	200	182	3.50	4.622222222	10.2	0.35708927	3.5411054
4	600	250	509	9.79	12.92698413	20	1.95818185	8.2116654
6	800	275	665	12.79	16.88888889	29.5	3.77353954	13.587921
8	1040	290	873	16.79	22.17142857	42	7.05291708	18.370225
10	1497	300	1169	22.49	29.68888889	55	12.367515	22.005371
12	1680	305	1321	25.41	33.54920635	65.5	16.6436802	24.517706
14	1730	308	1404	27.01	35.65714286	78	21.0652663	26.157258
16	1727	307	1419	27.30	36.03809524	72.5	19.7890819	27.190972
18	1721	306	1429	27.49	36.29206349	72	19.7911016	27.829368
20	1720	300	1430	27.51	36.31746032	71	19.5298825	28.218764

Appendix B4: Experimental biogas production at T=40 ^{o}C and pH=6.5 (R5)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	0.41743853
2	315	200	115	2.21	2.920634921	7	0.1548464	1.864649
4	560	250	310	5.96	7.873015873	20.5	1.22242099	4.7834548
6	672	275	397	7.64	10.08253968	31.3	2.39023217	8.6552026
8	900	290	610	11.73	15.49206349	39	4.57614404	12.571372
10	1135	300	835	16.06	21.20634921	56	8.99455633	15.900829
12	1280	305	975	18.75	24.76190476	61	11.4403601	18.435159
14	1415	308	1107	21.29	28.11428571	62	13.2021467	20.233665
16	1445	307	1138	21.89	28.9015873	59	12.9151519	21.454664
18	1448	306	1142	21.97	29.0031746	58.5	12.8507127	22.26073
20	1440	300	1140	21.93	28.95238095	56	12.2799931	22.783566

Appendix B5: Experimental biogas production at T=28 ^{o}C and pH=7.25 (R6)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	0.88215482
2	380	200	180	3.46	4.571428571	8.2	0.28391713	2.8031128
4	600	250	350	6.73	8.888888889	21	1.41381499	5.9208034
6	760	275	485	9.33	12.31746032	30	2.79877662	9.6031235
8	910	290	620	11.93	15.74603175	40.3	4.80620155	13.129695
10	1100	300	800	15.39	20.31746032	55	8.46365438	16.0736
12	1300	305	995	19.14	25.26984127	60	11.4836401	18.320501
14	1420	308	1112	21.39	28.24126984	62.5	13.3687268	19.938361
16	1425	307	1118	21.51	28.39365079	59	12.688172	21.06006
18	1422	306	1116	21.47	28.34285714	56	12.0214669	21.818928
20	1410	300	1110	21.35	28.19047619	55	11.7433204	22.324241

Appendix B6: Experimental biogas production at T=35 ^{o}C and pH=8.3 (R8)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	1.1704092
2	400	200	200	3.847115625	5.079365079	13.8	27.6	4.0176527
4	750	250	500	9.617789063	12.6984127	20	100	8.7612317
6	1048	275	773	14.86910189	19.63174603	28.5	220.305	14.341716
8	1272	290	982	18.88933772	24.93968254	35.2	345.664	19.583958
10	1500	300	1200	23.08269375	30.47619048	50.3	603.6	23.846671
12	1700	305	1395	26.83363148	35.42857143	68.5	955.575	27.008001
14	1861	308	1553	29.87285283	39.44126984	84.7	1315.391	29.219217
16	1946	307	1639	31.52711255	41.62539683	80.5	1319.395	30.709479
18	1950	306	1644	31.62329044	41.75238095	80	1315.2	31.690493
20	1950	300	1650	31.73870391	41.9047619	72	1188	32.326729

Appendix B7: Experimental biogas production at T=30 ^{o}C and pH=6.5 (R10)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	0.19715343
2	295	200	95	1.83	2.412698413	6.8	0.12426183	1.3809113
4	540	250	290	5.58	7.365079365	19	1.05988035	4.3412769
6	652	275	377	7.25	9.574603175	31	2.24806202	8.5180897
8	900	290	610	11.73	15.49206349	40	4.69348106	12.664721
10	1150	300	850	16.35	21.58730159	55	8.99263277	15.994031
12	1290	305	985	18.95	25.01587302	60	11.3682267	18.348662
14	1400	308	1092	21.01	27.73333333	60.5	12.708177	19.893138
16	1402	307	1095	21.06	27.80952381	58	12.2165157	20.862059
18	1399	306	1093	21.02	27.75873016	58	12.1942024	21.454131
20	1385	300	1085	20.87	27.55555556	55	11.4788312	21.810358

Appendix B7: Experimental biogas production at T=30 ^{o}C and pH=6.5 (R11)

Time (day)	Biogas (ml)	Control (ml)	Biogas Corrected (ml)	Biogas (ml/g COD)	Biogas (ml/g VS)	CH_4 comp (%)	CH4 volume (ml/g COD)	Biogas Predicted (ml/g COD)
0	0	0	0	0	0	0	0	0.29123551
2	309	200	109	2.10	2.768253968	12.1	0.25369804	1.6132946
4	520	250	270	5.19	6.857142857	22.3	1.15817416	4.6179725
6	700	275	425	8.18	10.79365079	32	2.61603863	8.8113061
8	960	290	670	12.89	17.01587302	40	5.15513494	13.104344
10	1150	300	850	16.35	21.58730159	54.3	8.87818108	16.722874
12	1300	305	995	19.14	25.26984127	79.3	15.1775444	19.425226
14	1500	308	1192	22.93	30.27301587	76	17.4258949	21.297646
16	1510	307	1203	23.14	30.55238095	70	16.1982803	22.536359
18	1499	306	1193	22.95	30.2984127	70.2	16.1095274	23.332804
20	1490	300	1190	22.89	30.22222222	70.6	16.1605786	23.835981

Appendix C 1: Dry basis molasses derived vinasse composition

Table B-1. Dry basis vinasse composition (molasses derived vinasse)

Component	As Received	Dry Basis
	%	%
Solids	29.79	n.a
Ash	13.31	18.95
Sulphur	0.08	0.12
Volatile matter	48.67	69.31
Fixed carbon	8.24	11.73
Carbon	n.a	39.72
Hydrogen	n.a	8.6
Nitrogen	n.a	1.65

Source: Cortez, L.A.B., L.E. Brossard Perez, Experiences on Vinasse disposal, Part III: Combustion of Vinasse -#6 Fuel Oil, Brazilian Journal of Chemical Engineering, Vol. 14, No. 1, 1997, São Paulo, Brazil.

n.a. means not available

Appendix C 2: Dry basis molasses derived vinasse composition

Table B – 2: Analysis of vinasse from different feed stocks [72]

	Sugar source					
	Cane juice	Cane molasses	Grapes (wine)	Agave (tequila)	Sweet sorghum	Beet molasses
BOD ($g\,L^{-1}$)	16.7	39.5	14.54–16.3	20.6	46	27.5–44.9
COD ($g\,L^{-1}$)	30.4	84.9–95	26–50.2	55.2–66.3	79.9	55.5–91.1
N_T ($mg\,L^{-1}$)	102–628	153–1230	104.9–650	na	800	1800–4750
P_T ($mg\,L^{-1}$)	71–130	1–190	65–118.4	41	1990	160–163
K ($mg\,L^{-1}$)	1733–1952	4893–11000	118–800	240–345	na	10000–10030
S_T ($mg\,L^{-1}$)	1356	1500–3480	120	780–880	na	3500–3720
pH	4.04–4.6	4.46–4.8	3–4.2	3.4	4.5	4.3–5.35
Cu ($mg\,L^{-1}$)	4	0.27–1.71	0.2–3.26	0.36–4	37	2.1–5*
Cd ($mg\,L^{-1}$)	na	0.04–1.36	0.05–0.08	0.01–0.2	na	<1*
Pb ($mg\,L^{-1}$)	na	0.02–0.48	0.55–1.34	0.065–0.5	na	<5*
Fe ($mg\,L^{-1}$)	16	12.8–157.5	0.001–0.077	35.2–45	317	203–226*
Phenols ($mg\,L^{-1}$)	na	34	29–474	44–81	na	450*
VY($LL^{-1}_{ethanol}$)	13	12–20	11.6	10–12	14.3–16	9–15
Reference	1	2	3	4	5	6

N_T, total nitrogen; P_T, total phosphorus; S_T, total sulfate; VY, vinasse yield; na, data not available.
*Unit is $mg\,kg^{-1}$.
Reference 1: Baez-Smith (2006); Wilkie et al. (2000); Salomon and Lora (2009); Cail and Barford (1985a).
Reference 2: Baez-Smith (2006); Wilkie et al. (2000); Pathak et al. (1999); Kannan and Upreti (2008); Chindankumar et al. (2009); Hutnan et al. (2003).
Reference 3: Bustamante et al. (2005); Vlyssides et al. (2005); Wilkie et al. (2000).
Reference 4: Ilangovan et al. (1997); CIATEJ (2005); Orendain (2006); Mendez-Acosta et al. (2010); Buitrón and Carvajal (2010); López et al. (2010).
Reference 5: Wilkie et al. (2000); Gnansounou et al. (2005); Cail and Barford (1985b).
Reference 6: Wilkie et al. (2000); Jiménez et al. (2006); Hutnan et al. (2003); Decloux et al. (2002); Tejada and Gonzalez (2006); Madejón et al. (2001)

Appendix D: Discharge Limit Values for Discharges to Water Malting, Brewing, Distilling, the Production of Wines and Other Alcoholic liquors

[75]

Constituent Group or Parameter	Emission Limit Value (mg/l)
Temperature	40 °C
pH	6 – 9 pH units
BOD_5 at 20°C	>90% Removal or 60 mg/l
COD	>90% Removal or 250 mg/l
Suspended Solids	50
Total Ammonia (as N)	20
Total Nitrogen (as N)	>80% Removal or 40 mg/l
Total Phosphorus (as P)	>80% Removal or 5 mg/l
Oils, Fats, and Grease	15
Mineral Oil (Interceptor)	20